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(54) Title: HUMAN CYTOKINE RECEPTOR

(57) Abstract: Cytokines and their receptors have proven usefulness in both basic research and as therapeutics. The present invention provides a new human cytokine receptor designated as "Zcytor21".

HUMAN CYTOKINE RECEPTOR

TECHNICAL FIELD

The present invention relates generally to a new protein expressed by human cells. In particular, the present invention relates to a novel gene that encodes a receptor, designated as "Zcytor21," and to nucleic acid molecules encoding Zcytor21 polypeptides.

BACKGROUND OF THE INVENTION

Cytokines are soluble, small proteins that mediate a variety of biological effects, including the regulation of the growth and differentiation of many cell types (see, for example, Arai *et al., Annu. Rev. Biochem. 59*:783 (1990); Mosmann, *Curr. Opin. Immunol. 3*:311 (1991); Paul and Seder, *Cell 76*:241 (1994)). Proteins that constitute the cytokine group include interleukins, interferons, colony stimulating factors, tumor necrosis factors, and other regulatory molecules. For example, human interleukin-17 is a cytokine which stimulates the expression of interleukin-6, intracellular adhesion molecule 1, interleukin-8, granulocyte macrophage colony-stimulating factor, and prostaglandin E2 expression, and plays a role in the preferential maturation of CD34+ hematopoietic precursors into neutrophils (Yao *et al., J. Immunol. 155*:5483 (1995); Fossiez *et al., J. Exp. Med. 183*:2593 (1996)).

Receptors that bind cytokines are typically composed of one or more integral membrane proteins that bind the cytokine with high affinity and transduce this binding event to the cell through the cytoplasmic portions of the certain receptor subunits. Cytokine receptors have been grouped into several classes on the basis of similarities in their extracellular ligand binding domains. For example, the receptor chains responsible for binding and/or transducing the effect of interferons are members of the type II cytokine receptor family, based upon a characteristic 200 residue extracellular domain.

The demonstrated *in vivo* activities of cytokines and their receptors illustrate the clinical potential of, and need for, other cytokines, cytokine receptors, cytokine agonists, and cytokine antagonists.

SUMMARY OF THE INVENTION

The present invention provides isolated polypeptides comprising an amino acid sequence that is at least 70%, at least 80%, or at least 90% identical to a reference amino acid sequence selected from the group consisting of: (a) amino acid residues 24 to 667 of SEQ ID NO:11, (b) amino acid residues 24 to 589 of SEQ ID NO:2, (c) amino acid residues 24 to 609 of SEQ ID NO:5, (d) amino acid residues 24 to 533 of SEQ ID NO:8, (e) amino acid residues 24 to 657 of SEQ ID NO:15, (f) amino acid residues 24 to 454 of SEQ ID NO:11, (g) amino acid residues 24 to 376 of SEQ ID NO:2, (h) amino acid residues 24 to 396 of SEQ ID NO:5, or (i) amino acid residues 24 to 444 of SEQ ID NO:15. Certain of these polypeptides can specifically bind with an antibody that specifically binds with a polypeptide consisting of the amino acid sequence of SEQ ID NOs:2, 5, 8, 11, or 15. Illustrative polypeptides include polypeptides comprising, or consisting of, amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 444 of SEQ ID NO:15, or amino acid residues 24 to 533 of SEQ ID NO:8.

The present invention also provides isolated polypeptides comprising at least 15 contiguous amino acid residues of an amino acid sequence selected from the group consisting of: (a) amino acid residues 24 to 667 of SEQ ID NO:11, (b) amino acid residues 24 to 589 of SEQ ID NO:2, (c) amino acid residues 24 to 609 of SEQ ID NO:5, (d) amino acid residues 24 to 533 of SEQ ID NO:8, (e) amino acid residues 24 to 657 of SEQ ID NO:15, (f) amino acid residues 24 to 454 of SEQ ID NO:11, (g) amino acid residues 24 to 376 of SEQ ID NO:2, (h) amino acid residues 24 to 396 of SEQ ID NO:5, or (i) amino acid residues 24 to 444 of SEQ ID NO:15. The present invention further provides isolated polypeptides comprising at least 30 contiguous amino acid residues of an amino acid sequence selected from the group consisting of: (a) amino acid residues 24 to 667 of SEQ ID NO:11, (b) amino acid residues 24 to 589 of SEQ ID NO:2, (c) amino

acid residues 24 to 609 of SEQ ID NO:5, (d) amino acid residues 24 to 533 of SEQ ID NO:8, (e) amino acid residues 24 to 657 of SEQ ID NO:15, (f) amino acid residues 24 to 454 of SEQ ID NO:11, (g) amino acid residues 24 to 376 of SEQ ID NO:2, (h) amino acid residues 24 to 396 of SEQ ID NO:5, or (i) amino acid residues 24 to 444 of SEQ ID NO:15. Illustrative polypeptides include polypeptides that either comprise, or consist of, amino acid residues (a) to (i).

The present invention also includes variant Zcytor21 polypeptides, wherein the amino acid sequence of the variant polypeptide shares a sequence identity with amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444, and wherein any difference between the amino acid sequence of the variant polypeptide and the corresponding amino acid sequence of SEQ ID NOs:2, 5, 8, 11, or 15, may be due to one or more conservative amino acid substitutions. The sequence identity may be at least 70% sequence identity, at least 80% sequence identity, at least sequence 90% identity, at least 95% sequence identity, or greater than 95% sequence identity.

The present invention further provides antibodies and antibody fragments that specifically bind with such polypeptides. Exemplary antibodies include polyclonal antibodies, murine monoclonal antibodies, humanized antibodies derived from murine monoclonal antibodies, and human monoclonal antibodies. Illustrative antibody fragments include $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv, and minimal recognition units. The present invention further includes compositions comprising a carrier and a peptide, polypeptide, or antibody described herein.

The present invention also provides isolated nucleic acid molecules that encode a Zcytor21 polypeptide, wherein the nucleic acid molecule is selected from the group consisting of: (a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOs:3, 6, 9, 12, or 16, (b) a nucleic acid molecule encoding an amino acid sequence that comprises amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444 of SEQ ID NO:15, and (c) a nucleic acid molecule that remains hybridized following stringent

wash conditions to a nucleic acid molecule comprising the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10, the nucleotide sequence of nucleotides 135 to 1427 of SEQ ID NO:10, the complement of the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10, or the complement of the nucleotide sequence of nucleotides 135 to 1427 of SEQ ID NO:10. Illustrative nucleic acid molecules include those in which any difference between the amino acid sequence encoded by nucleic acid molecule (c) and the corresponding amino acid sequence of SEQ ID NO:11 is due to a conservative amino acid substitution. Similarly, nucleic acid molecules used for hybridization can be derived from the nucleotide sequences of SEQ ID NOs: 1, 4, 7, or 14.

The present invention also includes vectors and expression vectors comprising such nucleic acid molecules. Such expression vectors may comprise a transcription promoter, and a transcription terminator, wherein the promoter is operably linked with the nucleic acid molecule, and wherein the nucleic acid molecule is operably linked with the transcription terminator. The present invention further includes recombinant host cells and recombinant viruses comprising these vectors and expression vectors. Illustrative host cells include avian, bacterial, yeast, fungal, insect, mammalian, and plant cells. Recombinant host cells comprising such expression vectors can be used to produce Zcytor21 polypeptides by culturing such recombinant host cells that comprise the expression vector and that produce the Zcytor21 protein, and, optionally, isolating the Zcytor21 protein from the cultured recombinant host cells. The present invention also provides polypeptide products produced by such methods.

In addition, the present invention provides pharmaceutical compositions comprising a pharmaceutically acceptable carrier and at least one of such an expression vector or recombinant virus comprising such expression vectors. The present invention further includes pharmaceutical compositions, comprising a pharmaceutically acceptable carrier and a polypeptide described herein.

The present invention also contemplates methods for detecting the presence of *Zcytor21* RNA in a biological sample, comprising the steps of (a) contacting a *Zcytor21* nucleic acid probe under hybridizing conditions with either (i) test RNA molecules isolated from the biological sample, or (ii) nucleic acid molecules synthesized

from the isolated RNA molecules, wherein the probe has a nucleotide sequence comprising a portion of the nucleotide sequence of SEQ ID NO:1, or its complement, and (b) detecting the formation of hybrids of the nucleic acid probe and either the test RNA molecules or the synthesized nucleic acid molecules following stringent wash conditions, wherein the presence of the hybrids indicates the presence of *Zcytor21* RNA in the biological sample. For example, suitable probes can consist of the following nucleotide sequences: nucleic acid molecules consisting of the nucleotide sequence of SEQ ID NO:10, nucleic acid molecules consisting of the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10, nucleotide sequence of nucleotides 135 to 1427 of SEQ ID NO:10, and the like. Other suitable probes consist of the complement of these nucleotide sequences, or a portion of the nucleotide sequences or their complements.

The present invention further provides methods for detecting the presence of Zcytor21 polypeptide in a biological sample, comprising the steps of: (a) contacting the biological sample with an antibody or an antibody fragment that specifically binds with a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NOs:2, 5, 8, 11, or 15 wherein the contacting is performed under conditions that allow the binding of the antibody or antibody fragment to the biological sample, and (b) detecting any of the bound antibody or bound antibody fragment. Such an antibody or antibody fragment may further comprise a detectable label selected from the group consisting of radioisotope, fluorescent label, chemiluminescent label, enzyme label, bioluminescent label, and colloidal gold.

The present invention also provides kits for performing these detection methods. For example, a kit for detection of *Zcytor21* gene expression may comprise a container that comprises a nucleic acid molecule, wherein the nucleic acid molecule is selected from the group consisting of (a) a nucleic acid molecule comprising the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10, (b) a nucleic acid molecule comprising the complement of nucleotides 66 to 2066 of the nucleotide sequence of SEQ ID NO:10, (c) a nucleic acid molecule comprising the nucleotide sequence of nucleotides 135 to 1427 of SEQ ID NO:10, (d) a nucleic acid molecule comprising the complement of nucleotides 135 to 1427 of the nucleotide sequence of

SEQ ID NO:10, and (e) a nucleic acid molecule that is a fragment of (a)-(d) consisting of at least eight nucleotides. Such a kit may also comprise a second container that comprises one or more reagents capable of indicating the presence of the nucleic acid molecule. On the other hand, a kit for detection of Zcytor21 protein may comprise a container that comprises an antibody, or an antibody fragment, that specifically binds with a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NOs:2, 5, 8, 11, or 15.

The present invention also contemplates anti-idiotype antibodies, or anti-idiotype antibody fragments, that specifically bind an antibody or antibody fragment that specifically binds a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NOs:2, 5, 8, 11, or 15. An exemplary anti-idiotype antibody binds with an antibody that specifically binds a polypeptide consisting of amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444 of SEQ ID NO:15.

The present invention also provides isolated nucleic acid molecules comprising a nucleotide sequence that encodes a Zcytor21 secretion signal sequence and a nucleotide sequence that encodes a biologically active polypeptide, wherein the Zcytor21 secretion signal sequence comprises an amino acid sequence of residues 1 to 23 of SEQ ID NO:2. Illustrative biologically active polypeptides include Factor VIIa, proinsulin, insulin, follicle stimulating hormone, tissue type plasminogen activator, tumor necrosis factor, interleukin, colony stimulating factor, interferon, erythropoietin, and thrombopoietin. Moreover, the present invention provides fusion proteins comprising a Zcytor21 secretion signal sequence and a polypeptide, wherein the Zcytor21 secretion signal sequence comprises an amino acid sequence of residues 1 to 23 of SEQ ID NO:2.

The present invention also provides fusion proteins, comprising a Zcytor21 polypeptide and an immunoglobulin moiety. Illustrative Zcytor21 polypeptides include polypeptides comprising amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444

of SEQ ID NO:15. In such fusion proteins, the immunoglobulin moiety may be an immunoglobulin heavy chain constant region, such as a human F_C fragment. The present invention further includes isolated nucleic acid molecules that encode such fusion proteins.

These and other aspects of the invention will become evident upon reference to the following detailed description.

DETAILED DESCRIPTION OF THE INVENTION

1. Overview

The present invention provides a novel receptor, designated "Zcytor21." The present invention also provides Zcytor21 polypeptides and Zcytor21 fusion proteins, as well as nucleic acid molecules encoding such polypeptides and proteins, and methods for using these nucleic acid molecules and amino acid sequences.

Four splice variants of Zcytor21 have been identified. The variants have been designated as Zcytor21-d2, Zcytor21-f1, Zcytor21-f5, Zcytor21-f6, and Zcytor21-g13. Table 1 provides the corresponding sequence identification numbers for the nucleotide and amino acid sequences of the variants, while Table 2 shows structural features of the Zcytor21 polypeptides. The *Zcytor21* gene resides in human chromosome 3p25.3.

Table 1

Zcytor21 Type	Sequence Type		
	Nucleotide	Amino Acid	Degenerate
Zcytor21-f1	1	2	3
Zcytor21-f5	4	5	6
Zcytor21-f6	7	8	9
Zcytor21-d2	10	11	12
Zcytor21-g13	14	15	16

Table 2

Protein	Location of Structural Features (amino acid residues within sequence)			
	Signal Sequence	Extracellular Domain	Transmembrane Domain	Intracellular Domain
Zcytor21-d2 (SEQ ID NO:11)	1-23	24-454	455-477	478-667
Zcytor21-f1 (SEQ ID NO:2)	1-23	24-376	377-399	400-589
Zcytor21-f5 (SEQ ID NO:5)	1-23	24-396	397-419	420-609
Zcytor21-f6 (SEQ ID NO:8)	1-23	24-533		
Zcytor21-g13 (SEQ ID NO:15)	1-23	24-444	445-467	468-657

The *Zcytor21* gene is expressed by human germ cell tumors and human skin cells. PCR analyses show that *Zcytor21* mRNA is present in human brain tissue, and tracheal tissue. In contrast, little or no evidence of gene expression was detected in adrenal gland, skeletal muscle, bladder, kidney, lung, and spleen. Thus, *Zcytor21* nucleotide and amino acid sequences can be used to differentiate tissues.

2. *Definitions*

In the description that follows, a number of terms are used extensively. The following definitions are provided to facilitate understanding of the invention.

Unless otherwise specified, "a," "an," "the," and "at least one" are used interchangeably and mean one or more than one.

As used herein, "nucleic acid" or "nucleic acid molecule" refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and

fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (*e.g.*, α-enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term "nucleic acid molecule" also includes so-called "peptide nucleic acids," which comprise naturally-occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded.

The term "complement of a nucleic acid molecule" refers to a nucleic acid molecule having a complementary nucleotide sequence and reverse orientation as compared to a reference nucleotide sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "contig" denotes a nucleic acid molecule that has a contiguous stretch of identical or complementary sequence to another nucleic acid molecule. Contiguous sequences are said to "overlap" a given stretch of a nucleic acid molecule either in their entirety or along a partial stretch of the nucleic acid molecule.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons as compared to a reference nucleic acid molecule that encodes a polypeptide. Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (*i.e.*, GAU and GAC triplets each encode Asp).

The term "structural gene" refers to a nucleic acid molecule that is transcribed into messenger RNA (mRNA), which is then translated into a sequence of amino acids characteristic of a specific polypeptide.

An "isolated nucleic acid molecule" is a nucleic acid molecule that is not integrated in the genomic DNA of an organism. For example, a DNA molecule that encodes a growth factor that has been separated from the genomic DNA of a cell is an isolated DNA molecule. Another example of an isolated nucleic acid molecule is a chemically-synthesized nucleic acid molecule that is not integrated in the genome of an organism. A nucleic acid molecule that has been isolated from a particular species is smaller than the complete DNA molecule of a chromosome from that species.

A "nucleic acid molecule construct" is a nucleic acid molecule, either single- or double-stranded, that has been modified through human intervention to contain segments of nucleic acid combined and juxtaposed in an arrangement not existing in nature.

"Linear DNA" denotes non-circular DNA molecules having free 5' and 3' ends. Linear DNA can be prepared from closed circular DNA molecules, such as plasmids, by enzymatic digestion or physical disruption.

"Complementary DNA (cDNA)" is a single-stranded DNA molecule that is formed from an mRNA template by the enzyme reverse transcriptase. Typically, a primer complementary to portions of mRNA is employed for the initiation of reverse transcription. Those skilled in the art also use the term "cDNA" to refer to a double-stranded DNA molecule consisting of such a single-stranded DNA molecule and its complementary DNA strand. The term "cDNA" also refers to a clone of a cDNA molecule synthesized from an RNA template.

A "promoter" is a nucleotide sequence that directs the transcription of a structural gene. Typically, a promoter is located in the 5' non-coding region of a gene, proximal to the transcriptional start site of a structural gene. Sequence elements within promoters that function in the initiation of transcription are often characterized by consensus nucleotide sequences. These promoter elements include RNA polymerase binding sites, TATA sequences, CAAT sequences, differentiation-specific elements (DSEs; McGehee *et al.*, *Mol. Endocrinol.* 7:551 (1993)), cyclic AMP response elements

(CREs), serum response elements (SREs; Treisman, *Seminars in Cancer Biol. 1*:47 (1990)), glucocorticoid response elements (GREs), and binding sites for other transcription factors, such as CRE/ATF (O'Reilly *et al., J. Biol. Chem. 267*:19938 (1992)), AP2 (Ye *et al., J. Biol. Chem. 269*:25728 (1994)), SP1, cAMP response element binding protein (CREB; Loeken, *Gene Expr. 3*:253 (1993)) and octamer factors (see, in general, Watson *et al.*, eds., *Molecular Biology of the Gene*, 4th ed. (The Benjamin/Cummings Publishing Company, Inc. 1987), and Lemaigre and Rousseau, *Biochem. J. 303*:1 (1994)). If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter. Repressible promoters are also known.

A "core promoter" contains essential nucleotide sequences for promoter function, including the TATA box and start of transcription. By this definition, a core promoter may or may not have detectable activity in the absence of specific sequences that may enhance the activity or confer tissue specific activity.

A "regulatory element" is a nucleotide sequence that modulates the activity of a core promoter. For example, a regulatory element may contain a nucleotide sequence that binds with cellular factors enabling transcription exclusively or preferentially in particular cells, tissues, or organelles. These types of regulatory elements are normally associated with genes that are expressed in a "cell-specific," "tissue-specific," or "organelle-specific" manner.

An "enhancer" is a type of regulatory element that can increase the efficiency of transcription, regardless of the distance or orientation of the enhancer relative to the start site of transcription.

"Heterologous DNA" refers to a DNA molecule, or a population of DNA molecules, that does not exist naturally within a given host cell. DNA molecules heterologous to a particular host cell may contain DNA derived from the host cell species (*i.e.,* endogenous DNA) so long as that host DNA is combined with non-host DNA (*i.e.,* exogenous DNA). For example, a DNA molecule containing a non-host DNA segment encoding a polypeptide operably linked to a host DNA segment comprising a transcription promoter is considered to be a heterologous DNA molecule.

Conversely, a heterologous DNA molecule can comprise an endogenous gene operably linked with an exogenous promoter. As another illustration, a DNA molecule comprising a gene derived from a wild-type cell is considered to be heterologous DNA if that DNA molecule is introduced into a mutant cell that lacks the wild-type gene.

A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides."

A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

A peptide or polypeptide encoded by a non-host DNA molecule is a "heterologous" peptide or polypeptide.

An "integrated genetic element" is a segment of DNA that has been incorporated into a chromosome of a host cell after that element is introduced into the cell through human manipulation. Within the present invention, integrated genetic elements are most commonly derived from linearized plasmids that are introduced into the cells by electroporation or other techniques. Integrated genetic elements are passed from the original host cell to its progeny.

A "cloning vector" is a nucleic acid molecule, such as a plasmid, cosmid, or bacteriophage, which has the capability of replicating autonomously in a host cell. Cloning vectors typically contain one or a small number of restriction endonuclease recognition sites that allow insertion of a nucleic acid molecule in a determinable fashion without loss of an essential biological function of the vector, as well as nucleotide sequences encoding a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Marker genes typically include genes that provide tetracycline resistance or ampicillin resistance.

An "expression vector" is a nucleic acid molecule encoding a gene that is expressed in a host cell. Typically, an expression vector comprises a transcription

promoter, a gene, and a transcription terminator. Gene expression is usually placed under the control of a promoter, and such a gene is said to be "operably linked to" the promoter. Similarly, a regulatory element and a core promoter are operably linked if the regulatory element modulates the activity of the core promoter.

A "recombinant host" is a cell that contains a heterologous nucleic acid molecule, such as a cloning vector or expression vector. In the present context, an example of a recombinant host is a cell that produces Zcytor21 from an expression vector. In contrast, Zcytor21 can be produced by a cell that is a "natural source" of Zcytor21, and that lacks an expression vector.

"Integrative transformants" are recombinant host cells, in which heterologous DNA has become integrated into the genomic DNA of the cells.

A "fusion protein" is a hybrid protein expressed by a nucleic acid molecule comprising nucleotide sequences of at least two genes. For example, a fusion protein can comprise at least part of a Zcytor21 polypeptide fused with a polypeptide that binds an affinity matrix. Such a fusion protein provides a means to isolate large quantities of Zcytor21 using affinity chromatography.

The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule termed a "ligand." This interaction mediates the effect of the ligand on the cell. Receptors can be membrane bound, cytosolic or nuclear; monomeric (*e.g.*, thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (*e.g.*, PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor). Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. In certain membrane-bound receptors, the extracellular ligand-binding domain and the intracellular effector domain are located in separate polypeptides that comprise the complete functional receptor.

In general, the binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell, which in turn leads to an alteration in the metabolism of the cell. Metabolic events that are often linked to receptor-ligand interactions include gene

transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids.

The term "secretory signal sequence" denotes a DNA sequence that encodes a peptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

An "isolated polypeptide" is a polypeptide that is essentially free from contaminating cellular components, such as carbohydrate, lipid, or other proteinaceous impurities associated with the polypeptide in nature. Typically, a preparation of isolated polypeptide contains the polypeptide in a highly purified form, *i.e.,* at least about 80% pure, at least about 90% pure, at least about 95% pure, greater than 95% pure, or greater than 99% pure. One way to show that a particular protein preparation contains an isolated polypeptide is by the appearance of a single band following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the protein preparation and Coomassie Brilliant Blue staining of the gel. However, the term "isolated" does not exclude the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

The term "expression" refers to the biosynthesis of a gene product. For example, in the case of a structural gene, expression involves transcription of the structural gene into mRNA and the translation of mRNA into one or more polypeptides.

The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative

splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a polypeptide encoded by a splice variant of an mRNA transcribed from a gene.

As used herein, the term "immunomodulator" includes cytokines, stem cell growth factors, lymphotoxins, co-stimulatory molecules, hematopoietic factors, and synthetic analogs of these molecules.

The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of less than 10^9 M^{-1}.

An "anti-idiotype antibody" is an antibody that binds with the variable region domain of an immunoglobulin. In the present context, an anti-idiotype antibody binds with the variable region of an anti-Zcytor21 antibody, and thus, an anti-idiotype antibody mimics an epitope of Zcytor21.

An "antibody fragment" is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. For example, an anti-Zcytor21 monoclonal antibody fragment binds with an epitope of Zcytor21.

The term "antibody fragment" also includes a synthetic or a genetically engineered polypeptide that binds to a specific antigen, such as polypeptides consisting of the light chain variable region, "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker ("scFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region.

A "chimeric antibody" is a recombinant protein that contains the variable domains and complementary determining regions derived from a rodent antibody, while the remainder of the antibody molecule is derived from a human antibody.

"Humanized antibodies" are recombinant proteins in which murine complementarity determining regions of a monoclonal antibody have been transferred from heavy and light variable chains of the murine immunoglobulin into a human variable domain.

As used herein, a "therapeutic agent" is a molecule or atom, which is conjugated to an antibody moiety to produce a conjugate, which is useful for therapy. Examples of therapeutic agents include drugs, toxins, immunomodulators, chelators, boron compounds, photoactive agents or dyes, and radioisotopes.

A "detectable label" is a molecule or atom, which can be conjugated to an antibody moiety to produce a molecule useful for diagnosis. Examples of detectable labels include chelators, photoactive agents, radioisotopes, fluorescent agents, paramagnetic ions, or other marker moieties.

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A (Nilsson *et al., EMBO J. 4*:1075 (1985); Nilsson *et al., Methods Enzymol. 198*:3 (1991)), glutathione S transferase (Smith and Johnson, *Gene 67*:31 (1988)), Glu-Glu affinity tag (Grussenmeyer *et al., Proc. Natl. Acad. Sci. USA 82*:7952 (1985)), substance P, FLAG peptide (Hopp *et al., Biotechnology 6*:1204 (1988)), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford *et al., Protein Expression and Purification 2*:95 (1991). DNA molecules encoding affinity tags are available from commercial suppliers (*e.g.*, Pharmacia Biotech, Piscataway, NJ).

A "naked antibody" is an entire antibody, as opposed to an antibody fragment, which is not conjugated with a therapeutic agent. Naked antibodies include

both polyclonal and monoclonal antibodies, as well as certain recombinant antibodies, such as chimeric and humanized antibodies.

As used herein, the term "antibody component" includes both an entire antibody and an antibody fragment.

An "immunoconjugate" is a conjugate of an antibody component with a therapeutic agent or a detectable label.

As used herein, the term "antibody fusion protein" refers to a recombinant molecule that comprises an antibody component and a Zcytor21 polypeptide component. Examples of an antibody fusion protein include a protein that comprises a Zcytor21 extracellular domain, and either an Fc domain or an antigen-biding region.

A "target polypeptide" or a "target peptide" is an amino acid sequence that comprises at least one epitope, and that is expressed on a target cell, such as a tumor cell, or a cell that carries an infectious agent antigen. T cells recognize peptide epitopes presented by a major histocompatibility complex molecule to a target polypeptide or target peptide and typically lyse the target cell or recruit other immune cells to the site of the target cell, thereby killing the target cell.

An "antigenic peptide" is a peptide, which will bind a major histocompatibility complex molecule to form an MHC-peptide complex, which is recognized by a T cell, thereby inducing a cytotoxic lymphocyte response upon presentation to the T cell. Thus, antigenic peptides are capable of binding to an appropriate major histocompatibility complex molecule and inducing a cytotoxic T cells response, such as cell lysis or specific cytokine release against the target cell, which binds or expresses the antigen. The antigenic peptide can be bound in the context of a class I or class II major histocompatibility complex molecule, on an antigen presenting cell or on a target cell.

In eukaryotes, RNA polymerase II catalyzes the transcription of a structural gene to produce mRNA. A nucleic acid molecule can be designed to contain an RNA polymerase II template in which the RNA transcript has a sequence that is complementary to that of a specific mRNA. The RNA transcript is termed an "anti-sense RNA" and a nucleic acid molecule that encodes the anti-sense RNA is termed an

"anti-sense gene." Anti-sense RNA molecules are capable of binding to mRNA molecules, resulting in an inhibition of mRNA translation.

An "anti-sense oligonucleotide specific for Zcytor21" or a "Zcytor21 anti-sense oligonucleotide" is an oligonucleotide having a sequence (a) capable of forming a stable triplex with a portion of the *Zcytor21* gene, or (b) capable of forming a stable duplex with a portion of an mRNA transcript of the *Zcytor21* gene.

A "ribozyme" is a nucleic acid molecule that contains a catalytic center. The term includes RNA enzymes, self-splicing RNAs, self-cleaving RNAs, and nucleic acid molecules that perform these catalytic functions. A nucleic acid molecule that encodes a ribozyme is termed a "ribozyme gene."

An "external guide sequence" is a nucleic acid molecule that directs the endogenous ribozyme, RNase P, to a particular species of intracellular mRNA, resulting in the cleavage of the mRNA by RNase P. A nucleic acid molecule that encodes an external guide sequence is termed an "external guide sequence gene."

The term "variant *Zcytor21* gene" refers to nucleic acid molecules that encode a polypeptide having an amino acid sequence that is a modification of SEQ ID NO:11. Such variants include naturally-occurring polymorphisms of *Zcytor21* genes, as well as synthetic genes that contain conservative amino acid substitutions of the amino acid sequence of SEQ ID NO:11. Additional variant forms of *Zcytor21* genes are nucleic acid molecules that contain insertions or deletions of the nucleotide sequences described herein. A variant *Zcytor21* gene can be identified, for example, by determining whether the gene hybridizes with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:10, or its complement, under stringent conditions.

Alternatively, variant *Zcytor21* genes can be identified by sequence comparison. Two amino acid sequences have "100% amino acid sequence identity" if the amino acid residues of the two amino acid sequences are the same when aligned for maximal correspondence. Similarly, two nucleotide sequences have "100% nucleotide sequence identity" if the nucleotide residues of the two nucleotide sequences are the same when aligned for maximal correspondence. Sequence comparisons can be performed using standard software programs such as those included in the LASERGENE bioinformatics computing suite, which is produced by DNASTAR (Madison,

Wisconsin). Other methods for comparing two nucleotide or amino acid sequences by determining optimal alignment are well-known to those of skill in the art (see, for example, Peruski and Peruski, *The Internet and the New Biology: Tools for Genomic and Molecular Research* (ASM Press, Inc. 1997), Wu *et al.* (eds.), "Information Superhighway and Computer Databases of Nucleic Acids and Proteins," in *Methods in Gene Biotechnology*, pages 123-151 (CRC Press, Inc. 1997), and Bishop (ed.), *Guide to Human Genome Computing*, 2nd Edition (Academic Press, Inc. 1998)). Particular methods for determining sequence identity are described below.

Regardless of the particular method used to identify a variant *Zcytor21* gene or variant Zcytor21 polypeptide, a variant gene or polypeptide encoded by a variant gene may be functionally characterized the ability to bind specifically to an anti-Zcytor21 antibody.

The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

The term "ortholog" denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

"Paralogs" are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, α-globin, β-globin, and myoglobin are paralogs of each other.

The present invention includes functional fragments of *Zcytor21* genes. Within the context of this invention, a "functional fragment" of a *Zcytor21* gene refers to a nucleic acid molecule that encodes a portion of a Zcytor21 polypeptide, which is a domain described herein or at least specifically binds with an anti-Zcytor21 antibody.

Due to the imprecision of standard analytical methods, molecular weights and lengths of polymers are understood to be approximate values. When such a value is

expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to ±10%.

3. Production of Nucleic Acid Molecules That Encode Zcytor21

Nucleic acid molecules encoding a human Zcytor21 can be obtained by screening a human cDNA or genomic library using polynucleotide probes based upon any of SEQ ID NOs:1, 4, 7, 10, 14. These techniques are standard and well-established.

As an illustration, a nucleic acid molecule that encodes human Zcytor21 can be isolated from a cDNA library. In this case, the first step would be to prepare the cDNA library by isolating RNA from a tissue, such as skin tissue, using methods well-known to those of skill in the art. In general, RNA isolation techniques must provide a method for breaking cells, a means of inhibiting RNase-directed degradation of RNA, and a method of separating RNA from DNA, protein, and polysaccharide contaminants. For example, total RNA can be isolated by freezing tissue in liquid nitrogen, grinding the frozen tissue with a mortar and pestle to lyse the cells, extracting the ground tissue with a solution of phenol/chloroform to remove proteins, and separating RNA from the remaining impurities by selective precipitation with lithium chloride (see, for example, Ausubel *et al.* (eds.), *Short Protocols in Molecular Biology, 3rd Edition*, pages 4-1 to 4-6 (John Wiley & Sons 1995) ["Ausubel (1995)"]; Wu *et al.*, *Methods in Gene Biotechnology*, pages 33-41 (CRC Press, Inc. 1997) ["Wu (1997)"]).

Alternatively, total RNA can be isolated by extracting ground tissue with guanidinium isothiocyanate, extracting with organic solvents, and separating RNA from contaminants using differential centrifugation (see, for example, Chirgwin *et al., Biochemistry 18*:52 (1979); Ausubel (1995) at pages 4-1 to 4-6; Wu (1997) at pages 33-41).

In order to construct a cDNA library, poly(A)$^+$ RNA must be isolated from a total RNA preparation. Poly(A)$^+$ RNA can be isolated from total RNA using the standard technique of oligo(dT)-cellulose chromatography (see, for example, Aviv and Leder, *Proc. Nat'l Acad. Sci. USA 69:*1408 (1972); Ausubel (1995) at pages 4-11 to 4-12).

Double-stranded cDNA molecules are synthesized from poly(A)$^+$ RNA using techniques well-known to those in the art. (see, for example, Wu (1997) at pages

41-46). Moreover, commercially available kits can be used to synthesize double-stranded cDNA molecules. For example, such kits are available from Life Technologies, Inc. (Gaithersburg, MD), CLONTECH Laboratories, Inc. (Palo Alto, CA), Promega Corporation (Madison, WI) and STRATAGENE (La Jolla, CA).

Various cloning vectors are appropriate for the construction of a cDNA library. For example, a cDNA library can be prepared in a vector derived from bacteriophage, such as a λgt10 vector. See, for example, Huynh *et al.*, "Constructing and Screening cDNA Libraries in λgt10 and λgt11," in *DNA Cloning: A Practical Approach Vol. I*, Glover (ed.), page 49 (IRL Press, 1985); Wu (1997) at pages 47-52.

Alternatively, double-stranded cDNA molecules can be inserted into a plasmid vector, such as a PBLUESCRIPT vector (STRATAGENE; La Jolla, CA), a LAMDAGEM-4 (Promega Corp.) or other commercially available vectors. Suitable cloning vectors also can be obtained from the American Type Culture Collection (Manassas, VA).

To amplify the cloned cDNA molecules, the cDNA library is inserted into a prokaryotic host, using standard techniques. For example, a cDNA library can be introduced into competent *E. coli* DH5 cells, which can be obtained, for example, from Life Technologies, Inc. (Gaithersburg, MD).

A human genomic library can be prepared by means well-known in the art (see, for example, Ausubel (1995) at pages 5-1 to 5-6; Wu (1997) at pages 307-327). Genomic DNA can be isolated by lysing tissue with the detergent Sarkosyl, digesting the lysate with proteinase K, clearing insoluble debris from the lysate by centrifugation, precipitating nucleic acid from the lysate using isopropanol, and purifying resuspended DNA on a cesium chloride density gradient.

DNA fragments that are suitable for the production of a genomic library can be obtained by the random shearing of genomic DNA or by the partial digestion of genomic DNA with restriction endonucleases. Genomic DNA fragments can be inserted into a vector, such as a bacteriophage or cosmid vector, in accordance with conventional techniques, such as the use of restriction enzyme digestion to provide appropriate termini, the use of alkaline phosphatase treatment to avoid undesirable joining of DNA molecules,

and ligation with appropriate ligases. Techniques for such manipulation are well-known in the art (see, for example, Ausubel (1995) at pages 5-1 to 5-6; Wu (1997) at pages 307-327).

Alternatively, human genomic libraries can be obtained from commercial sources such as Research Genetics (Huntsville, AL) and the American Type Culture Collection (Manassas, VA).

A library containing cDNA or genomic clones can be screened with one or more polynucleotide probes based upon SEQ ID NOs:1, 4, 7, 10, or 14, using standard methods (see, for example, Ausubel (1995) at pages 6-1 to 6-11).

Nucleic acid molecules that encode a human *Zcytor21* gene can also be obtained using the polymerase chain reaction (PCR) with oligonucleotide primers having nucleotide sequences that are based upon the nucleotide sequences of the *Zcytor21* gene, as described herein. General methods for screening libraries with PCR are provided by, for example, Yu *et al.*, "Use of the Polymerase Chain Reaction to Screen Phage Libraries," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications*, White (ed.), pages 211-215 (Humana Press, Inc. 1993). Moreover, techniques for using PCR to isolate related genes are described by, for example, Preston, "Use of Degenerate Oligonucleotide Primers and the Polymerase Chain Reaction to Clone Gene Family Members," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications*, White (ed.), pages 317-337 (Humana Press, Inc. 1993).

Anti-Zcytor21 antibodies, produced as described below, can also be used to isolate DNA sequences that encode human *Zcytor21* genes from cDNA libraries. For example, the antibodies can be used to screen λgt11 expression libraries, or the antibodies can be used for immunoscreening following hybrid selection and translation (see, for example, Ausubel (1995) at pages 6-12 to 6-16; Margolis *et al.*, "Screening λ expression libraries with antibody and protein probes," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 1-14 (Oxford University Press 1995)).

As an alternative, a *Zcytor21* gene can be obtained by synthesizing nucleic acid molecules using mutually priming long oligonucleotides and the nucleotide sequences described herein (see, for example, Ausubel (1995) at pages 8-8 to 8-9). Established techniques using the polymerase chain reaction provide the ability to

synthesize DNA molecules at least two kilobases in length (Adang *et al.*, *Plant Molec. Biol. 21*:1131 (1993), Bambot *et al.*, *PCR Methods and Applications 2*:266 (1993), Dillon *et al.*, "Use of the Polymerase Chain Reaction for the Rapid Construction of Synthetic Genes," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications*, White (ed.), pages 263-268, (Humana Press, Inc. 1993), and Holowachuk *et al., PCR Methods Appl. 4*:299 (1995)).

The nucleic acid molecules of the present invention can also be synthesized with "gene machines" using protocols such as the phosphoramidite method. If chemically-synthesized double stranded DNA is required for an application such as the synthesis of a gene or a gene fragment, then each complementary strand is made separately. The production of short genes (60 to 80 base pairs) is technically straightforward and can be accomplished by synthesizing the complementary strands and then annealing them. For the production of longer genes (>300 base pairs), however, special strategies may be required, because the coupling efficiency of each cycle during chemical DNA synthesis is seldom 100%. To overcome this problem, synthetic genes (double-stranded) are assembled in modular form from single-stranded fragments that are from 20 to 100 nucleotides in length. For reviews on polynucleotide synthesis, see, for example, Glick and Pasternak, *Molecular Biotechnology, Principles and Applications of Recombinant DNA* (ASM Press 1994), Itakura *et al., Annu. Rev. Biochem. 53*:323 (1984), and Climie *et al., Proc. Nat'l Acad. Sci. USA 87*:633 (1990).

The sequence of a *Zcytor21* cDNA or *Zcytor21* genomic fragment can be determined using standard methods. Zcytor21 polynucleotide sequences disclosed herein can also be used as probes or primers to clone 5' non-coding regions of a *Zcytor21* gene. Promoter elements from a *Zcytor21* gene can be used to direct the expression of heterologous genes in, for example, transgenic animals or patients treated with gene therapy. The identification of genomic fragments containing a *Zcytor21* promoter or regulatory element can be achieved using well-established techniques, such as deletion analysis (see, generally, Ausubel (1995)).

Cloning of 5' flanking sequences also facilitates production of Zcytor21 proteins by "gene activation," as disclosed in U.S. Patent No. 5,641,670. Briefly, expression of an endogenous *Zcytor21* gene in a cell is altered by introducing into the

Zcytor21 locus a DNA construct comprising at least a targeting sequence, a regulatory sequence, an exon, and an unpaired splice donor site. The targeting sequence is a *Zcytor21* 5' non-coding sequence that permits homologous recombination of the construct with the endogenous *Zcytor21* locus, whereby the sequences within the construct become operably linked with the endogenous *Zcytor21* coding sequence. In this way, an endogenous *Zcytor21* promoter can be replaced or supplemented with other regulatory sequences to provide enhanced, tissue-specific, or otherwise regulated expression.

4. *Production of Zcytor21 Variants*

The present invention provides a variety of nucleic acid molecules, including DNA and RNA molecules, which encode the Zcytor21 polypeptides disclosed herein. Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. For example, SEQ ID NO:12 is a degenerate nucleotide sequence that encompasses all nucleic acid molecules that encode the Zcytor21-d2 polypeptide of SEQ ID NO:11. Those skilled in the art will recognize that the degenerate sequence of SEQ ID NO:12 also provides all RNA sequences encoding SEQ ID NO:11, by substituting U for T. Thus, the present invention contemplates Zcytor21-d2 polypeptide-encoding nucleic acid molecules comprising nucleotide 66 to nucleotide 2066 of SEQ ID NO:10, and their RNA equivalents. Similarly, the present invention contemplates Zcytor21 polypeptide-encoding nucleic acid molecules comprising nucleotide sequences that encode Zcytor21-f1, Zcytor21-f5, Zcytor21-f6, Zcytor21-g13, and their RNA equivalents.

Table 3 sets forth the one-letter codes used within SEQ ID NOs:3, 6, 9, 12, and 16 to denote degenerate nucleotide positions. "Resolutions" are the nucleotides denoted by a code letter. "Complement" indicates the code for the complementary nucleotide(s). For example, the code Y denotes either C or T, and its complement R denotes A or G, A being complementary to T, and G being complementary to C.

Table 3

Nucleotide	Resolution	Complement	Resolution
A	A	T	T
C	C	G	G
G	G	C	C
T	T	A	A
R	A|G	Y	C|T
Y	C|T	R	A|G
M	A|C	K	G|T
K	G|T	M	A|C
S	C|G	S	C|G
W	A|T	W	A|T
H	A|C|T	D	A|G|T
B	C|G|T	V	A|C|G
V	A|C|G	B	C|G|T
D	A|G|T	H	A|C|T
N	A|C|G|T	N	A|C|G|T

The degenerate codons used in SEQ ID NOs:3, 6, 9, 12, and 16, encompassing all possible codons for a given amino acid, are set forth in Table 4.

Table 4

Amino Acid	One Letter Code	Codons	Degenerate Codon
Cys	C	TGC TGT	TGY
Ser	S	AGC AGT TCA TCC TCG TCT	WSN
Thr	T	ACA ACC ACG ACT	ACN
Pro	P	CCA CCC CCG CCT	CCN
Ala	A	GCA GCC GCG GCT	GCN
Gly	G	GGA GGC GGG GGT	GGN
Asn	N	AAC AAT	AAY
Asp	D	GAC GAT	GAY
Glu	E	GAA GAG	GAR
Gln	Q	CAA CAG	CAR
His	H	CAC CAT	CAY
Arg	R	AGA AGG CGA CGC CGG CGT	MGN
Lys	K	AAA AAG	AAR
Met	M	ATG	ATG
Ile	I	ATA ATC ATT	ATH
Leu	L	CTA CTC CTG CTT TTA TTG	YTN
Val	V	GTA GTC GTG GTT	GTN
Phe	F	TTC TTT	TTY
Tyr	Y	TAC TAT	TAY
Trp	W	TGG	TGG
Ter	.	TAA TAG TGA	TRR
Asn|Asp	B		RAY
Glu|Gln	Z		SAR
Any	X		NNN

One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding an amino acid. For example, the degenerate codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine (MGN) can, in some circumstances, encode serine (AGY). A similar relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequences of SEQ ID NOs:2, 5, 8, 11, and 15. Variant sequences can be readily tested for functionality as described herein.

Different species can exhibit "preferential codon usage." In general, see, Grantham *et al., Nucl. Acids Res. 8*:1893 (1980), Haas *et al. Curr. Biol. 6*:315 (1996), Wain-Hobson *et al., Gene 13*:355 (1981), Grosjean and Fiers, *Gene 18*:199 (1982), Holm, *Nuc. Acids Res. 14:*3075 (1986), Ikemura, *J. Mol. Biol. 158*:573 (1982), Sharp and Matassi, *Curr. Opin. Genet. Dev. 4*:851 (1994), Kane, *Curr. Opin. Biotechnol. 6*:494 (1995), and Makrides, *Microbiol. Rev. 60*:512 (1996). As used herein, the term "preferential codon usage" or "preferential codons" is a term of art referring to protein translation codons that are most frequently used in cells of a certain species, thus favoring one or a few representatives of the possible codons encoding each amino acid (See Table 4). For example, the amino acid threonine (Thr) may be encoded by ACA, ACC, ACG, or ACT, but in mammalian cells ACC is the most commonly used codon; in other species, for example, insect cells, yeast, viruses or bacteria, different Thr codons may be preferential. Preferential codons for a particular species can be introduced into the polynucleotides of the present invention by a variety of methods known in the art. Introduction of preferential codon sequences into recombinant DNA can, for example, enhance production of the protein by making protein translation more efficient within a particular cell type or species. Therefore, the degenerate codon sequences disclosed herein serve as a template for optimizing expression of polynucleotides in various cell types and species commonly used in the art and disclosed herein. Sequences containing preferential codons can be tested and optimized for expression in various species, and tested for functionality as disclosed herein.

The present invention further provides variant polypeptides and nucleic acid molecules that represent counterparts from other species (orthologs). These species include, but are not limited to mammalian, avian, amphibian, reptile, fish, insect and other vertebrate and invertebrate species. Of particular interest are Zcytor21 polypeptides from other mammalian species, including mouse, porcine, ovine, bovine, canine, feline, equine, and other primate polypeptides. Orthologs of human Zcytor21 can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a *Zcytor21* cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses *Zcytor21* as disclosed herein. Suitable sources of mRNA can be identified by probing northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line.

A *Zcytor21*-encoding cDNA can be isolated by a variety of methods, such as by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction with primers designed from the representative human *Zcytor21* sequences disclosed herein. In addition, a cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to Zcytor21 polypeptide.

Those skilled in the art will recognize that the sequence disclosed in SEQ ID NO:10 represents a single allele of human *Zcytor21*, and that allelic variation and alternative splicing are expected to occur. Allelic variants of this sequence can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. Allelic variants of the nucleotide sequences disclosed herein, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of the amino acid sequences disclosed herein. cDNA molecules generated from alternatively spliced mRNAs, which retain the properties of the Zcytor21 polypeptide are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants

of these sequences can be cloned by probing cDNA or genomic libraries from different individuals or tissues according to standard procedures known in the art.

Within certain embodiments of the invention, the isolated nucleic acid molecules can hybridize under stringent conditions to nucleic acid molecules comprising nucleotide sequences disclosed herein. Using Zcytor21-d2 as an example, such nucleic acid molecules can hybridize under stringent conditions to nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:10, to nucleic acid molecules consisting of the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10, nucleotide sequence of nucleotides 135 to 1427 of SEQ ID NO:10, or to nucleic acid molecules comprising a nucleotide sequence complementary any of the nucleotide sequence of SEQ ID NO:10, the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10, or nucleotides 135 to 1427 of SEQ ID NO:10. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe.

A pair of nucleic acid molecules, such as DNA-DNA, RNA-RNA and DNA-RNA, can hybridize if the nucleotide sequences have some degree of complementarity. Hybrids can tolerate mismatched base pairs in the double helix, but the stability of the hybrid is influenced by the degree of mismatch. The T_m of the mismatched hybrid decreases by 1°C for every 1-1.5% base pair mismatch. Varying the stringency of the hybridization conditions allows control over the degree of mismatch that will be present in the hybrid. The degree of stringency increases as the hybridization temperature increases and the ionic strength of the hybridization buffer decreases. Stringent hybridization conditions encompass temperatures of about 5°C - 25°C below the T_m of the hybrid and a hybridization buffer having up to 1 M Na^+. Higher degrees of stringency at lower temperatures can be achieved with the addition of formamide which reduces the T_m of the hybrid about 1°C for each 1% formamide in the buffer solution. Generally, such stringent conditions include temperatures of 20°C - 70°C and a hybridization buffer containing up to 6x SSC and 0-50% formamide. A higher degree of stringency can be achieved at temperatures of from 40°C - 70°C with a

hybridization buffer having up to 4x SSC and from 0-50% formamide. Highly stringent conditions typically encompass temperatures of 42°C - 70°C with a hybridization buffer having up to 1x SSC and 0-50% formamide. Different degrees of stringency can be used during hybridization and washing to achieve maximum specific binding to the target sequence. Typically, the washes following hybridization are performed at increasing degrees of stringency to remove non-hybridized polynucleotide probes from hybridized complexes.

The above conditions are meant to serve as a guide and it is well within the abilities of one skilled in the art to adapt these conditions for use with a particular polypeptide hybrid. The T_m for a specific target sequence is the temperature (under defined conditions) at which 50% of the target sequence will hybridize to a perfectly matched probe sequence. Conditions that influence the T_m include, the size and base pair content of the polynucleotide probe, the ionic strength of the hybridization solution, and the presence of destabilizing agents in the hybridization solution. Numerous equations for calculating T_m are known in the art, and are specific for DNA, RNA and DNA-RNA hybrids and polynucleotide probe sequences of varying length (see, for example, Sambrook *et al., Molecular Cloning: A Laboratory Manual*, Second Edition (Cold Spring Harbor Press 1989); Ausubel *et al.*, (eds.), *Current Protocols in Molecular Biology* (John Wiley and Sons, Inc. 1987); Berger and Kimmel (eds.), *Guide to Molecular Cloning Techniques*, (Academic Press, Inc. 1987); and Wetmur, *Crit. Rev. Biochem. Mol. Biol. 26*:227 (1990)). Sequence analysis software such as OLIGO 6.0 (LSR; Long Lake, MN) and *Primer Premier 4.0* (Premier Biosoft International; Palo Alto, CA), as well as sites on the Internet, are available tools for analyzing a given sequence and calculating T_m based on user defined criteria. Such programs can also analyze a given sequence under defined conditions and identify suitable probe sequences. Typically, hybridization of longer polynucleotide sequences, >50 base pairs, is performed at temperatures of about 20°C - 25°C below the calculated T_m. For smaller probes, <50 base pairs, hybridization is typically carried out at the T_m or 5°C - 10°C below. This allows for the maximum rate of hybridization for DNA-DNA and DNA-RNA hybrids.

The length of the polynucleotide sequence influences the rate and stability of hybrid formation. Smaller probe sequences, <50 base pairs, reach equilibrium with complementary sequences rapidly, but may form less stable hybrids. Incubation times of anywhere from minutes to hours can be used to achieve hybrid formation. Longer probe sequences come to equilibrium more slowly, but form more stable complexes even at lower temperatures. Incubations are allowed to proceed overnight or longer. Generally, incubations are carried out for a period equal to three times the calculated Cot time. Cot time, the time it takes for the polynucleotide sequences to reassociate, can be calculated for a particular sequence by methods known in the art.

The base pair composition of polynucleotide sequence will effect the thermal stability of the hybrid complex, thereby influencing the choice of hybridization temperature and the ionic strength of the hybridization buffer. A-T pairs are less stable than G-C pairs in aqueous solutions containing sodium chloride. Therefore, the higher the G-C content, the more stable the hybrid. Even distribution of G and C residues within the sequence also contribute positively to hybrid stability. In addition, the base pair composition can be manipulated to alter the T_m of a given sequence. For example, 5-methyldeoxycytidine can be substituted for deoxycytidine and 5-bromodeoxuridine can be substituted for thymidine to increase the T_m, whereas 7-deazz-2'-deoxyguanosine can be substituted for guanosine to reduce dependence on T_m.

The ionic concentration of the hybridization buffer also affects the stability of the hybrid. Hybridization buffers generally contain blocking agents such as Denhardt's solution (Sigma Chemical Co., St. Louis, Mo.), denatured salmon sperm DNA, tRNA, milk powders (BLOTTO), heparin or SDS, and a Na^+ source, such as SSC (1x SSC: 0.15 M sodium chloride, 15 mM sodium citrate) or SSPE (1x SSPE: 1.8 M NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.7). Typically, hybridization buffers contain from between 10 mM - 1 M Na^+. The addition of destabilizing or denaturing agents such as formamide, tetralkylammonium salts, guanidinium cations or thiocyanate cations to the hybridization solution will alter the T_m of a hybrid. Typically, formamide is used at a concentration of up to 50% to allow incubations to be

carried out at more convenient and lower temperatures. Formamide also acts to reduce non-specific background when using RNA probes.

As an illustration, a nucleic acid molecule encoding a variant Zcytor21 polypeptide can be hybridized with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:10 (or its complement) at 42°C overnight in a solution comprising 50% formamide, 5x SSC, 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution (100x Denhardt's solution: 2% (w/v) Ficoll 400, 2% (w/v) polyvinylpyrrolidone, and 2% (w/v) bovine serum albumin), 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA. One of skill in the art can devise variations of these hybridization conditions. For example, the hybridization mixture can be incubated at a higher temperature, such as about 65°C, in a solution that does not contain formamide. Moreover, premixed hybridization solutions are available (*e.g.*, EXPRESSHYB Hybridization Solution from CLONTECH Laboratories, Inc.), and hybridization can be performed according to the manufacturer's instructions.

Following hybridization, the nucleic acid molecules can be washed to remove non-hybridized nucleic acid molecules under stringent conditions, or under highly stringent conditions. Typical stringent washing conditions include washing in a solution of 0.5x - 2x SSC with 0.1% sodium dodecyl sulfate (SDS) at 55°C - 65°C. As an illustration, nucleic acid molecules encoding a variant Zcytor21-d2 polypeptide remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10 (or its complement) under stringent washing conditions, in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55°C - 65°C, including 0.5x SSC with 0.1% SDS at 55°C, or 2xSSC with 0.1% SDS at 65°C. One of skill in the art can readily devise equivalent conditions, for example, by substituting SSPE for SSC in the wash solution.

Typical highly stringent washing conditions include washing in a solution of 0.1x - 0.2x SSC with 0.1% sodium dodecyl sulfate (SDS) at 50°C - 65°C. For example, nucleic acid molecules encoding a variant Zcytor21-d2 polypeptide remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10 (or its complement) under highly stringent washing conditions, in which the wash stringency is equivalent to 0.1x - 0.2x SSC with

0.1% SDS at 50°C - 65°C, including 0.1x SSC with 0.1% SDS at 50°C, or 0.2xSSC with 0.1% SDS at 65°C.

The present invention also provides isolated Zcytor21 polypeptides that have a substantially similar sequence identity to the polypeptides of SEQ ID NOs:2, 5, 8, 11, and 15, or their orthologs. The term "substantially similar sequence identity" is used herein to denote polypeptides having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the sequences shown in SEQ ID NOs:2, 5, 8, 11, and 15, or their orthologs.

The present invention also contemplates *Zcytor21* variant nucleic acid molecules that can be identified using two criteria: a determination of the similarity between the encoded polypeptide with an amino acid sequence disclosed herein, and a hybridization assay, as described above. As an illustration, *Zcytor21-d2* variants include nucleic acid molecules (1) that remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10 (or its complement) under stringent washing conditions, in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55°C - 65°C, and (2) that encode a polypeptide having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the amino acid sequence of SEQ ID NO:11. Alternatively, *Zcytor21-d2* variants can be characterized as nucleic acid molecules (1) that remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10 (or its complement) under highly stringent washing conditions, in which the wash stringency is equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50°C - 65°C, and (2) that encode a polypeptide having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the amino acid sequence of SEQ ID NO:11.

Percent sequence identity is determined by conventional methods. See, for example, Altschul *et al., Bull. Math. Bio. 48*:603 (1986), and Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA 89*:10915 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM62" scoring matrix of Henikoff and Henikoff (*ibid.*) as shown in Table 5 (amino acids are indicated by the standard one-

letter codes). The percent identity is then calculated as: ([Total number of identical matches]/ [length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences])(100).

Table 5

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative Zcytor21 variant. The FASTA algorithm is described by Pearson and Lipman, *Proc. Nat'l Acad. Sci. USA 85*:2444 (1988), and by Pearson, *Meth. Enzymol. 183*:63 (1990). Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (*e.g.*, SEQ ID NO:11) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, *J. Mol. Biol. 48*:444 (1970); Sellers, *SIAM J. Appl. Math. 26*:787 (1974)), which allows for amino acid insertions and deletions. Illustrative parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol. 183*:63 (1990).

FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as described above.

The present invention includes nucleic acid molecules that encode a polypeptide having a conservative amino acid change, compared with an amino acid

sequence disclosed herein. For example, variants can be obtained that contain one or more amino acid substitutions of SEQ ID NOs:2, 5, 8, 11, or 15, in which an alkyl amino acid is substituted for an alkyl amino acid in a Zcytor21 amino acid sequence, an aromatic amino acid is substituted for an aromatic amino acid in a Zcytor21 amino acid sequence, a sulfur-containing amino acid is substituted for a sulfur-containing amino acid in a Zcytor21 amino acid sequence, a hydroxy-containing amino acid is substituted for a hydroxy-containing amino acid in a Zcytor21 amino acid sequence, an acidic amino acid is substituted for an acidic amino acid in a Zcytor21 amino acid sequence, a basic amino acid is substituted for a basic amino acid in a Zcytor21 amino acid sequence, or a dibasic monocarboxylic amino acid is substituted for a dibasic monocarboxylic amino acid in a Zcytor21 amino acid sequence. Among the common amino acids, for example, a "conservative amino acid substitution" is illustrated by a substitution among amino acids within each of the following groups: (1) glycine, alanine, valine, leucine, and isoleucine, (2) phenylalanine, tyrosine, and tryptophan, (3) serine and threonine, (4) aspartate and glutamate, (5) glutamine and asparagine, and (6) lysine, arginine and histidine.

The BLOSUM62 table is an amino acid substitution matrix derived from about 2,000 local multiple alignments of protein sequence segments, representing highly conserved regions of more than 500 groups of related proteins (Henikoff and Henikoff, *Proc. Nat'l Acad. Sci. USA 89*:10915 (1992)). Accordingly, the BLOSUM62 substitution frequencies can be used to define conservative amino acid substitutions that may be introduced into the amino acid sequences of the present invention. Although it is possible to design amino acid substitutions based solely upon chemical properties (as discussed above), the language "conservative amino acid substitution" preferably refers to a substitution represented by a BLOSUM62 value of greater than -1. For example, an amino acid substitution is conservative if the substitution is characterized by a BLOSUM62 value of 0, 1, 2, or 3. According to this system, preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 1 (*e.g.*, 1, 2 or 3), while more preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 2 (*e.g.*, 2 or 3).

Particular variants of Zcytor21 are characterized by having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the corresponding amino acid sequence (*e.g.*, SEQ ID NOs:2, 5, 8, 11, or 15), wherein the variation in amino acid sequence is due to one or more conservative amino acid substitutions.

Conservative amino acid changes in a *Zcytor21* gene can be introduced, for example, by substituting nucleotides for the nucleotides recited in SEQ ID NO:10. Such "conservative amino acid" variants can be obtained by oligonucleotide-directed mutagenesis, linker-scanning mutagenesis, mutagenesis using the polymerase chain reaction, and the like (see Ausubel (1995) at pages 8-10 to 8-22; and McPherson (ed.), *Directed Mutagenesis: A Practical Approach* (IRL Press 1991)). A variant Zcytor21 polypeptide can be identified by the ability to specifically bind anti-Zcytor21 antibodies.

The proteins of the present invention can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methylglycine, *allo*-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, *tert*-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is typically carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson *et al.*, *J. Am. Chem. Soc. 113*:2722 (1991), Ellman *et al.*, *Methods Enzymol. 202*:301 (1991), Chung *et al.*, *Science 259*:806 (1993), and Chung *et al.*, *Proc. Nat'l Acad. Sci. USA 90*:10145 (1993).

In a second method, translation is carried out in *Xenopus* oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti *et al., J. Biol. Chem. 271*:19991 (1996)). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (*e.g.*, phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (*e.g.*, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide *et al., Biochem. 33*:7470 (1994). Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, *Protein Sci. 2*:395 (1993)).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for Zcytor21 amino acid residues.

Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081 (1989), Bass *et al., Proc. Nat'l Acad. Sci. USA 88*:4498 (1991), Coombs and Corey, "Site-Directed Mutagenesis and Protein Engineering," in *Proteins: Analysis and Design*, Angeletti (ed.), pages 259-311 (Academic Press, Inc. 1998)). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al., J. Biol. Chem. 271*:4699 (1996).

Although sequence analysis can be used to further define the Zcytor21 ligand binding region, amino acids that play a role in Zcytor21 binding activity can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for

example, de Vos *et al., Science 255*:306 (1992), Smith *et al., J. Mol. Biol. 224*:899 (1992), and Wlodaver *et al., FEBS Lett. 309*:59 (1992).

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (*Science 241*:53 (1988)) or Bowie and Sauer (*Proc. Nat'l Acad. Sci. USA 86:*2152 (1989)). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (*e.g.*, Lowman *et al., Biochem. 30*:10832 (1991), Ladner *et al.*, U.S. Patent No. 5,223,409, Huse, international publication No. WO 92/06204, and region-directed mutagenesis (Derbyshire *et al., Gene 46*:145 (1986), and Ner *et al., DNA 7:*127, (1988)). Moreover, Zcytor21 labeled with biotin or FITC can be used for expression cloning of Zcytor21 ligands.

Variants of the disclosed Zcytor21 nucleotide and polypeptide sequences can also be generated through DNA shuffling as disclosed by Stemmer, *Nature 370*:389 (1994), Stemmer, *Proc. Nat'l Acad. Sci. USA 91*:10747 (1994), and international publication No. WO 97/20078. Briefly, variant DNA molecules are generated by *in vitro* homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNA molecules, such as allelic variants or DNA molecules from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid "evolution" of sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

Mutagenesis methods as disclosed herein can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode biologically active polypeptides, or polypeptides that bind with anti-Zcytor21 antibodies, can be recovered from the host cells and rapidly sequenced using modern equipment. These methods

allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

The present invention also includes "functional fragments" of Zcytor21 polypeptides and nucleic acid molecules encoding such functional fragments. Routine deletion analyses of nucleic acid molecules can be performed to obtain functional fragments of a nucleic acid molecule that encodes a Zcytor21 polypeptide. As an illustration, DNA molecules comprising the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10 can be digested with *Bal*31 nuclease to obtain a series of nested deletions. The fragments are then inserted into expression vectors in proper reading frame, and the expressed polypeptides are isolated and tested for the ability to bind anti-Zcytor21 antibodies. One alternative to exonuclease digestion is to use oligonucleotide-directed mutagenesis to introduce deletions or stop codons to specify production of a desired fragment. Alternatively, particular fragments of a *Zcytor21* gene can be synthesized using the polymerase chain reaction. An example of a functional fragment is the extracellular domain of Zcytor21 (*i.e.,* amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444 of SEQ ID NO:15).

This general approach is exemplified by studies on the truncation at either or both termini of interferons have been summarized by Horisberger and Di Marco, *Pharmac. Ther. 66*:507 (1995). Moreover, standard techniques for functional analysis of proteins are described by, for example, Treuter *et al., Molec. Gen. Genet. 240*:113 (1993), Content *et al.*, "Expression and preliminary deletion analysis of the 42 kDa 2-5A synthetase induced by human interferon," in *Biological Interferon Systems, Proceedings of ISIR-TNO Meeting on Interferon Systems*, Cantell (ed.), pages 65-72 (Nijhoff 1987), Herschman, "The EGF Receptor," in *Control of Animal Cell Proliferation, Vol. 1*, Boynton *et al.*, (eds.) pages 169-199 (Academic Press 1985), Coumailleau *et al., J. Biol. Chem. 270*:29270 (1995); Fukunaga *et al., J. Biol. Chem. 270*:25291 (1995); Yamaguchi *et al., Biochem. Pharmacol. 50*:1295 (1995), and Meisel *et al., Plant Molec. Biol. 30*:1 (1996).

The present invention also contemplates functional fragments of a *Zcytor21* gene that have amino acid changes, compared with an amino acid sequence disclosed herein. A variant *Zcytor21* gene can be identified on the basis of structure by determining the level of identity with disclosed nucleotide and amino acid sequences, as discussed above. An alternative approach to identifying a variant gene on the basis of structure is to determine whether a nucleic acid molecule encoding a potential variant *Zcytor21* gene can hybridize to a nucleic acid molecule comprising a *Zcytor21* nucleotide sequence, such as SEQ ID NO:10.

The present invention also provides polypeptide fragments or peptides comprising an epitope-bearing portion of a Zcytor21 polypeptide described herein. Such fragments or peptides may comprise an "immunogenic epitope," which is a part of a protein that elicits an antibody response when the entire protein is used as an immunogen. Immunogenic epitope-bearing peptides can be identified using standard methods (see, for example, Geysen *et al., Proc. Nat'l Acad. Sci. USA 81*:3998 (1983)).

In contrast, polypeptide fragments or peptides may comprise an "antigenic epitope," which is a region of a protein molecule to which an antibody can specifically bind. Certain epitopes consist of a linear or contiguous stretch of amino acids, and the antigenicity of such an epitope is not disrupted by denaturing agents. It is known in the art that relatively short synthetic peptides that can mimic epitopes of a protein can be used to stimulate the production of antibodies against the protein (see, for example, Sutcliffe *et al., Science 219*:660 (1983)). Accordingly, antigenic epitope-bearing peptides and polypeptides of the present invention are useful to raise antibodies that bind with the polypeptides described herein.

Antigenic epitope-bearing peptides and polypeptides can contain at least four to ten amino acids, at least ten to fifteen amino acids, or about 15 to about 30 amino acids of an amino acid sequence disclosed herein. Such epitope-bearing peptides and polypeptides can be produced by fragmenting a Zcytor21 polypeptide, or by chemical peptide synthesis, as described herein. Moreover, epitopes can be selected by phage display of random peptide libraries (see, for example, Lane and Stephen, *Curr. Opin. Immunol. 5*:268 (1993), and Cortese *et al., Curr. Opin. Biotechnol. 7*:616 (1996)). Standard methods for identifying epitopes and producing antibodies from

small peptides that comprise an epitope are described, for example, by Mole, "Epitope Mapping," in *Methods in Molecular Biology, Vol. 10*, Manson (ed.), pages 105-116 (The Humana Press, Inc. 1992), Price, "Production and Characterization of Synthetic Peptide-Derived Antibodies," in *Monoclonal Antibodies: Production, Engineering, and Clinical Application*, Ritter and Ladyman (eds.), pages 60-84 (Cambridge University Press 1995), and Coligan *et al.* (eds.), *Current Protocols in Immunology*, pages 9.3.1 - 9.3.5 and pages 9.4.1 - 9.4.11 (John Wiley & Sons 1997).

In addition to the uses described above, polynucleotides and polypeptides of the present invention are useful as educational tools in laboratory practicum kits for courses related to genetics and molecular biology, protein chemistry, and antibody production and analysis. Due to its unique polynucleotide and polypeptide sequences, molecules of Zcytor21 can be used as standards or as "unknowns" for testing purposes. Advantageously, the present invention provides four Zcytor21 variants suitable for analysis. As an illustration, Zcytor21 polynucleotides can be used as an aid, such as, for example, to teach a student how to prepare expression constructs for bacterial, viral, or mammalian expression, including fusion constructs, wherein Zcytor21 is the gene to be expressed; for determining the restriction endonuclease cleavage sites of the polynucleotides; determining mRNA and DNA localization of Zcytor21 polynucleotides in tissues (*i.e.*, by northern and Southern blotting as well as polymerase chain reaction); and for identifying related polynucleotides and polypeptides by nucleic acid hybridization. As an illustration, students will find that *Bam*HI digestion of a nucleic acid molecule consisting of the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10 provides two fragments of about 475 base pairs, and 1526 base pairs, and that *Xho*I digestion yields fragments of about 963 base pairs, and 1038 base pairs.

Zcytor21 polypeptides can be used as an aid to teach preparation of antibodies; identifying proteins by western blotting; protein purification; determining the weight of expressed Zcytor21 polypeptides as a ratio to total protein expressed; identifying peptide cleavage sites; coupling amino and carboxyl terminal tags; amino acid sequence analysis, as well as, but not limited to monitoring biological activities of both the native and tagged protein (*i.e.*, protease inhibition) *in vitro* and *in vivo*. For

example, students will find that digestion of unglycosylated Zcytor21 with hydroxylamine yields two fragments having approximate molecular weights of 36361, and 38465, whereas digestion of unglycosylated Zcytor21 with mild acid hydrolysis yields fragments having approximate molecular weights of 18042, 45959, and 10842.

Zcytor21 polypeptides can also be used to teach analytical skills such as mass spectrometry, circular dichroism, to determine conformation, especially of the four alpha helices, x-ray crystallography to determine the three-dimensional structure in atomic detail, nuclear magnetic resonance spectroscopy to reveal the structure of proteins in solution. For example, a kit containing the Zcytor21 can be given to the student to analyze. Since the amino acid sequence would be known by the instructor, the protein can be given to the student as a test to determine the skills or develop the skills of the student, the instructor would then know whether or not the student has correctly analyzed the polypeptide. Since every polypeptide is unique, the educational utility of Zcytor21 would be unique unto itself.

The antibodies which bind specifically to Zcytor21 can be used as a teaching aid to instruct students how to prepare affinity chromatography columns to purify Zcytor21, cloning and sequencing the polynucleotide that encodes an antibody and thus as a practicum for teaching a student how to design humanized antibodies. The *Zcytor21* gene, polypeptide, or antibody would then be packaged by reagent companies and sold to educational institutions so that the students gain skill in art of molecular biology. Because each gene and protein is unique, each gene and protein creates unique challenges and learning experiences for students in a lab practicum. Such educational kits containing the *Zcytor21* gene, polypeptide, or antibody are considered within the scope of the present invention.

For any Zcytor21 polypeptide, including variants and fusion proteins, one of ordinary skill in the art can readily generate a fully degenerate polynucleotide sequence encoding that variant using the information set forth in Tables 3 and 4 above. Moreover, those of skill in the art can use standard software to devise Zcytor21 variants based upon the nucleotide and amino acid sequences described herein. Accordingly, the present invention includes a computer-readable medium encoded with a data structure that provides at least one of the following sequences: SEQ ID NO:1, SEQ ID

NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16. Suitable forms of computer-readable media include magnetic media and optically-readable media. Examples of magnetic media include a hard or fixed drive, a random access memory (RAM) chip, a floppy disk, digital linear tape (DLT), a disk cache, and a ZIP disk. Optically readable media are exemplified by compact discs (*e.g.*, CD-read only memory (ROM), CD-rewritable (RW), and CD-recordable), and digital versatile/video discs (DVD) (*e.g.*, DVD-ROM, DVD-RAM, and DVD+RW).

5. *Production of Zcytor21 Polypeptides*

The polypeptides of the present invention, including full-length polypeptides, functional fragments, and fusion proteins, can be produced in recombinant host cells following conventional techniques. To express a *Zcytor21* gene, a nucleic acid molecule encoding the polypeptide must be operably linked to regulatory sequences that control transcriptional expression in an expression vector and then, introduced into a host cell. In addition to transcriptional regulatory sequences, such as promoters and enhancers, expression vectors can include translational regulatory sequences and a marker gene, which is suitable for selection of cells that carry the expression vector.

Expression vectors that are suitable for production of a foreign protein in eukaryotic cells typically contain (1) prokaryotic DNA elements coding for a bacterial replication origin and an antibiotic resistance marker to provide for the growth and selection of the expression vector in a bacterial host; (2) eukaryotic DNA elements that control initiation of transcription, such as a promoter; and (3) DNA elements that control the processing of transcripts, such as a transcription termination/polyadenylation sequence. As discussed above, expression vectors can also include nucleotide sequences encoding a secretory sequence that directs the heterologous polypeptide into the secretory pathway of a host cell. For example, a *Zcytor21* expression vector may comprise a *Zcytor21* gene and a secretory sequence derived from any secreted gene.

Zcytor21 proteins of the present invention may be expressed in mammalian cells. Examples of suitable mammalian host cells include African green

monkey kidney cells (Vero; ATCC CRL 1587), human embryonic kidney cells (293-HEK; ATCC CRL 1573), baby hamster kidney cells (BHK-21, BHK-570; ATCC CRL 8544, ATCC CRL 10314), canine kidney cells (MDCK; ATCC CCL 34), Chinese hamster ovary cells (CHO-K1; ATCC CCL61; CHO DG44 (Chasin *et al., Som. Cell. Molec. Genet. 12*:555, 1986)), rat pituitary cells (GH1; ATCC CCL82), HeLa S3 cells (ATCC CCL2.2), rat hepatoma cells (H-4-II-E; ATCC CRL 1548) SV40-transformed monkey kidney cells (COS-1; ATCC CRL 1650) and murine embryonic cells (NIH-3T3; ATCC CRL 1658).

For a mammalian host, the transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, simian virus, or the like, in which the regulatory signals are associated with a particular gene which has a high level of expression. Suitable transcriptional and translational regulatory sequences also can be obtained from mammalian genes, such as actin, collagen, myosin, and metallothionein genes.

Transcriptional regulatory sequences include a promoter region sufficient to direct the initiation of RNA synthesis. Suitable eukaryotic promoters include the promoter of the mouse *metallothionein I* gene (Hamer *et al., J. Molec. Appl. Genet. 1*:273 (1982)), the *TK* promoter of *Herpes* virus (McKnight, *Cell 31*:355 (1982)), the *SV40* early promoter (Benoist *et al., Nature 290*:304 (1981)), the *Rous* sarcoma virus promoter (Gorman *et al., Proc. Nat'l Acad. Sci. USA 79*:6777 (1982)), the cytomegalovirus promoter (Foecking *et al., Gene 45*:101 (1980)), and the mouse mammary tumor virus promoter (see, generally, Etcheverry, "Expression of Engineered Proteins in Mammalian Cell Culture," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 163-181 (John Wiley & Sons, Inc. 1996)).

Alternatively, a prokaryotic promoter, such as the bacteriophage T3 RNA polymerase promoter, can be used to control *Zcytor21* gene expression in mammalian cells if the prokaryotic promoter is regulated by a eukaryotic promoter (Zhou *et al., Mol. Cell. Biol. 10*:4529 (1990), and Kaufman *et al., Nucl. Acids Res. 19*:4485 (1991)).

An expression vector can be introduced into host cells using a variety of standard techniques including calcium phosphate transfection, liposome-mediated

transfection, microprojectile-mediated delivery, electroporation, and the like. The transfected cells can be selected and propagated to provide recombinant host cells that comprise the expression vector stably integrated in the host cell genome. Techniques for introducing vectors into eukaryotic cells and techniques for selecting such stable transformants using a dominant selectable marker are described, for example, by Ausubel (1995) and by Murray (ed.), *Gene Transfer and Expression Protocols* (Humana Press 1991).

For example, one suitable selectable marker is a gene that provides resistance to the antibiotic neomycin. In this case, selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A suitable amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (*e.g.*, hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Alternatively, markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

Zcytor21 polypeptides can also be produced by cultured mammalian cells using a viral delivery system. Exemplary viruses for this purpose include adenovirus, herpesvirus, vaccinia virus and adeno-associated virus (AAV). Adenovirus, a double-stranded DNA virus, is currently the best studied gene transfer vector for delivery of heterologous nucleic acid (for a review, see Becker *et al., Meth. Cell Biol. 43*:161 (1994), and Douglas and Curiel, *Science & Medicine 4:*44 (1997)). Advantages of the adenovirus system include the accommodation of relatively large DNA inserts, the ability to grow to high-titer, the ability to infect a broad range of mammalian cell types, and flexibility that allows use with a large number of available vectors containing different promoters.

By deleting portions of the adenovirus genome, larger inserts (up to 7 kb) of heterologous DNA can be accommodated. These inserts can be incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. An option is to delete the essential *E1* gene from the viral vector, which results in the inability to replicate unless the *E1* gene is provided by the host cell. Adenovirus vector-infected human 293 cells (ATCC Nos. CRL-1573, 45504, 45505), for example, can be grown as adherent cells or in suspension culture at relatively high cell density to produce significant amounts of protein (see Garnier *et al., Cytotechnol. 15*:145 (1994)).

Zcytor21 can also be expressed in other higher eukaryotic cells, such as avian, fungal, insect, yeast, or plant cells. The baculovirus system provides an efficient means to introduce cloned *Zcytor21* genes into insect cells. Suitable expression vectors are based upon the *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV), and contain well-known promoters such as *Drosophila heat shock protein* (*hsp*) 70 promoter, *Autographa californica nuclear polyhedrosis virus immediate-early* gene promoter (*ie-1*) and the *delayed early 39K* promoter, baculovirus *p10* promoter, and the *Drosophila metallothionein* promoter. A second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow (Luckow, *et al., J. Virol. 67*:4566 (1993)). This system, which utilizes transfer vectors, is sold in the BAC-to-BAC kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, PFASTBAC (Life Technologies) containing a Tn7 transposon to move the DNA encoding the Zcytor21 polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." See, Hill-Perkins and Possee, *J. Gen. Virol. 71*:971 (1990), Bonning, *et al., J. Gen. Virol. 75*:1551 (1994), and Chazenbalk, and Rapoport, *J. Biol. Chem. 270*:1543 (1995). In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed Zcytor21 polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer *et al., Proc. Nat'l Acad. Sci. 82*:7952 (1985)). Using a technique known in the art, a transfer vector containing a *Zcytor21* gene is transformed into *E. coli*, and screened for bacmids, which contain an interrupted *lacZ* gene indicative of recombinant baculovirus. The bacmid

DNA containing the recombinant baculovirus genome is then isolated using common techniques.

The illustrative PFASTBAC vector can be modified to a considerable degree. For example, the polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as P*cor*, p6.9 or MP promoter) which is expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins (see, for example, Hill-Perkins and Possee, *J. Gen. Virol. 71*:971 (1990), Bonning, *et al., J. Gen. Virol. 75*:1551 (1994), and Chazenbalk and Rapoport, *J. Biol. Chem. 270*:1543 (1995). In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed, which replace the native Zcytor21 secretory signal sequences with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen Corporation; Carlsbad, CA), or baculovirus gp67 (PharMingen: San Diego, CA) can be used in constructs to replace the native Zcytor21 secretory signal sequence.

The recombinant virus or bacmid is used to transfect host cells. Suitable insect host cells include cell lines derived from IPLB-*Sf*-21, a *Spodoptera frugiperda* pupal ovarian cell line, such as *Sf*9 (ATCC CRL 1711), *Sf*21AE, and *Sf*21 (Invitrogen Corporation; San Diego, CA), as well as *Drosophila* Schneider-2 cells, and the HIGH FIVEO cell line (Invitrogen) derived from *Trichoplusia ni* (U.S. Patent No. 5,300,435). Commercially available serum-free media can be used to grow and to maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cellO405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the *T. ni* cells. When recombinant virus is used, the cells are typically grown up from an inoculation density of approximately 2-5 x 10^5 cells to a density of 1-2 x 10^6 cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3.

Established techniques for producing recombinant proteins in baculovirus systems are provided by Bailey *et al.,* "Manipulation of Baculovirus Vectors," in *Methods in Molecular Biology, Volume 7: Gene Transfer and Expression*

Protocols, Murray (ed.), pages 147-168 (The Humana Press, Inc. 1991), by Patel *et al.*, "The baculovirus expression system," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 205-244 (Oxford University Press 1995), by Ausubel (1995) at pages 16-37 to 16-57, by Richardson (ed.), *Baculovirus Expression Protocols* (The Humana Press, Inc. 1995), and by Lucknow, "Insect Cell Expression Technology," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 183-218 (John Wiley & Sons, Inc. 1996).

Fungal cells, including yeast cells, can also be used to express the genes described herein. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Suitable promoters for expression in yeast include promoters from *GAL1* (galactose), *PGK* (phosphoglycerate kinase), *ADH* (alcohol dehydrogenase), *AOX1* (alcohol oxidase), HIS4 (histidinol dehydrogenase), and the like. Many yeast cloning vectors have been designed and are readily available. These vectors include YIp-based vectors, such as YIp5, YRp vectors, such as YRp17, YEp vectors such as YEp13 and YCp vectors, such as YCp19. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311, Kawasaki *et al.*, U.S. Patent No. 4,931,373, Brake, U.S. Patent No. 4,870,008, Welch *et al.*, U.S. Patent No. 5,037,743, and Murray *et al.*, U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (*e.g.*, leucine). A suitable vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki *et al.* (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Additional suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, *e.g.*, Kawasaki, U.S. Patent No. 4,599,311, Kingsman *et al.*, U.S. Patent No. 4,615,974, and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446, 5,063,154, 5,139,936, and 4,661,454.

Transformation systems for other yeasts, including *Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces*

fragilis, Ustilago maydis, Pichia pastoris, Pichia methanolica, Pichia guillermondii and *Candida maltosa* are known in the art. See, for example, Gleeson *et al., J. Gen. Microbiol. 132*:3459 (1986), and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight *et al.,* U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.,* U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

For example, the use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed by Raymond, U.S. Patent No. 5,716,808, Raymond, U.S. Patent No. 5,736,383, Raymond *et al., Yeast 14*:11-23 (1998), and in international publication Nos. WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming *P. methanolica* will commonly be prepared as double-stranded, circular plasmids, which are preferably linearized prior to transformation. For polypeptide production in *P. methanolica*, the promoter and terminator in the plasmid can be that of a *P. methanolica* gene, such as a *P. methanolica* alcohol utilization gene (*AUG1* or *AUG2*). Other useful promoters include those of the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, it is preferred to have the entire expression segment of the plasmid flanked at both ends by host DNA sequences. A suitable selectable marker for use in *Pichia methanolica* is a *P. methanolica ADE2* gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (AIRC; EC 4.1.1.21), and which allows *ade2* host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, host cells can be used in which both methanol utilization genes (*AUG1* and *AUG2*) are deleted. For production of secreted proteins, host cells can be deficient in vacuolar protease genes (*PEP4* and *PRB1*). Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into *P. methanolica* cells. *P. methanolica* cells can be transformed by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (t) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

Expression vectors can also be introduced into plant protoplasts, intact plant tissues, or isolated plant cells. Methods for introducing expression vectors into plant tissue include the direct infection or co-cultivation of plant tissue with *Agrobacterium tumefaciens*, microprojectile-mediated delivery, DNA injection, electroporation, and the like. See, for example, Horsch *et al., Science 227*:1229 (1985), Klein *et al., Biotechnology 10*:268 (1992), and Miki *et al.*, "Procedures for Introducing Foreign DNA into Plants," in *Methods in Plant Molecular Biology and Biotechnology*, Glick *et al.* (eds.), pages 67-88 (CRC Press, 1993).

Alternatively, *Zcytor21* genes can be expressed in prokaryotic host cells. Suitable promoters that can be used to express Zcytor21 polypeptides in a prokaryotic host are well-known to those of skill in the art and include promoters capable of recognizing the T4, T3, Sp6 and T7 polymerases, the P_R and P_L promoters of bacteriophage lambda, the *trp*, *recA*, heat shock, *lacUV5*, *tac*, *lpp-lacSpr*, *phoA*, and *lacZ* promoters of *E. coli*, promoters of *B. subtilis*, the promoters of the bacteriophages of *Bacillus*, *Streptomyces* promoters, the *int* promoter of bacteriophage lambda, the *bla* promoter of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene. Prokaryotic promoters have been reviewed by Glick, *J. Ind. Microbiol. 1*:277 (1987), Watson *et al., Molecular Biology of the Gene, 4th Ed.* (Benjamin Cummins 1987), and by Ausubel *et al.* (1995).

Suitable prokaryotic hosts include *E. coli* and *Bacillus subtilus*. Suitable strains of *E. coli* include BL21(DE3), BL21(DE3)pLysS, BL21(DE3)pLysE, DH1, DH4I, DH5, DH5I, DH5IF', DH5IMCR, DH10B, DH10B/p3, DH11S, C600, HB101, JM101, JM105, JM109, JM110, K38, RR1, Y1088, Y1089, CSH18, ER1451, and ER1647 (see, for example, Brown (ed.), *Molecular Biology Labfax* (Academic Press 1991)). Suitable strains of *Bacillus subtilus* include BR151, YB886, MI119, MI120, and B170 (see, for example, Hardy, "Bacillus Cloning Methods," in *DNA Cloning: A Practical Approach*, Glover (ed.) (IRL Press 1985)).

When expressing a Zcytor21 polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example,

guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.

Methods for expressing proteins in prokaryotic hosts are well-known to those of skill in the art (see, for example, Williams *et al.,* "Expression of foreign proteins in *E. coli* using plasmid vectors and purification of specific polyclonal antibodies," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), page 15 (Oxford University Press 1995), Ward *et al.,* "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, page 137 (Wiley-Liss, Inc. 1995), and Georgiou, "Expression of Proteins in Bacteria," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), page 101 (John Wiley & Sons, Inc. 1996)).

Standard methods for introducing expression vectors into bacterial, yeast, insect, and plant cells are provided, for example, by Ausubel (1995).

General methods for expressing and recovering foreign protein produced by a mammalian cell system are provided by, for example, Etcheverry, "Expression of Engineered Proteins in Mammalian Cell Culture," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 163 (Wiley-Liss, Inc. 1996). Standard techniques for recovering protein produced by a bacterial system is provided by, for example, Grisshammer *et al.*, "Purification of over-produced proteins from *E. coli* cells," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 59-92 (Oxford University Press 1995). Established methods for isolating recombinant proteins from a baculovirus system are described by Richardson (ed.), *Baculovirus Expression Protocols* (The Humana Press, Inc. 1995).

As an alternative, polypeptides of the present invention can be synthesized by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. These synthesis methods are well-known

to those of skill in the art (see, for example, Merrifield, *J. Am. Chem. Soc. 85*:2149 (1963), Stewart *et al.*, "Solid Phase Peptide Synthesis" (2nd Edition), (Pierce Chemical Co. 1984), Bayer and Rapp, *Chem. Pept. Prot. 3*:3 (1986), Atherton *et al., Solid Phase Peptide Synthesis: A Practical Approach* (IRL Press 1989), Fields and Colowick, "Solid-Phase Peptide Synthesis," *Methods in Enzymology Volume 289* (Academic Press 1997), and Lloyd-Williams *et al., Chemical Approaches to the Synthesis of Peptides and Proteins* (CRC Press, Inc. 1997)). Variations in total chemical synthesis strategies, such as "native chemical ligation" and "expressed protein ligation" are also standard (see, for example, Dawson *et al., Science 266*:776 (1994), Hackeng *et al., Proc. Nat'l Acad. Sci. USA 94*:7845 (1997), Dawson, *Methods Enzymol. 287*: 34 (1997), Muir *et al, Proc. Nat'l Acad. Sci. USA 95*:6705 (1998), and Severinov and Muir, *J. Biol. Chem. 273*:16205 (1998)).

Peptides and polypeptides of the present invention comprise at least six, at least nine, or at least 15 contiguous amino acid residues of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:15. As an illustration, polypeptides can comprise at least six, at least nine, or at least 15 contiguous amino acid residues of any of the following amino acid sequences: (a) amino acid residues 24 to 667 of SEQ ID NO:11, (b) amino acid residues 24 to 589 of SEQ ID NO:2, (c) amino acid residues 24 to 609 of SEQ ID NO:5, (d) amino acid residues 24 to 533 of SEQ ID NO:8, (e) amino acid residues 24 to 657 of SEQ ID NO:15, (f) amino acid residues 24 to 454 of SEQ ID NO:11, (g) amino acid residues 24 to 376 of SEQ ID NO:2, (h) amino acid residues 24 to 396 of SEQ ID NO:5, or (i) amino acid residues 24 to 444 of SEQ ID NO:15. Within certain embodiments of the invention, the polypeptides comprise 20, 30, 40, 50, 100, or more contiguous residues of these amino acid sequences. For example, polypeptides can comprise at least 30 contiguous amino acid residues of an amino acid sequence selected from the group consisting of: (a) amino acid residues 24 to 667 of SEQ ID NO:11, (b) amino acid residues 24 to 589 of SEQ ID NO:2, (c) amino acid residues 24 to 609 of SEQ ID NO:5, (d) amino acid residues 24 to 533 of SEQ ID NO:8, (e) amino acid residues 24 to 657 of SEQ ID NO:15, (f) amino acid residues 24 to 454 of SEQ ID NO:11, (g) amino acid residues 24 to 376 of SEQ ID NO:2, (h) amino acid residues 24 to 396 of SEQ ID NO:5, or (i) amino acid residues 24 to 444 of

SEQ ID NO:15. Nucleic acid molecules encoding such peptides and polypeptides are useful as polymerase chain reaction primers and probes.

6. Production of Zcytor21 Fusion Proteins and Conjugates

One general class of Zcytor21 analogs are variants having an amino acid sequence that is a mutation of the amino acid sequence disclosed herein. Another general class of Zcytor21 analogs is provided by anti-idiotype antibodies, and fragments thereof, as described below. Moreover, recombinant antibodies comprising anti-idiotype variable domains can be used as analogs (see, for example, Monfardini *et al., Proc. Assoc. Am. Physicians 108*:420 (1996)). Since the variable domains of anti-idiotype Zcytor21 antibodies mimic Zcytor21, these domains can provide Zcytor21 binding activity. Methods of producing anti-idiotypic catalytic antibodies are known to those of skill in the art (see, for example, Joron *et al., Ann. N Y Acad. Sci. 672*:216 (1992), Friboulet *et al., Appl. Biochem. Biotechnol. 47*:229 (1994), and Avalle *et al., Ann. N Y Acad. Sci. 864*:118 (1998)).

Another approach to identifying Zcytor21 analogs is provided by the use of combinatorial libraries. Methods for constructing and screening phage display and other combinatorial libraries are provided, for example, by Kay *et al., Phage Display of Peptides and Proteins* (Academic Press 1996), Verdine, U.S. Patent No. 5,783,384, Kay, *et. al.,* U.S. Patent No. 5,747,334, and Kauffman *et al.,* U.S. Patent No. 5,723,323.

Zcytor21 polypeptides have both *in vivo* and *in vitro* uses. As an illustration, a soluble form of Zcytor21 can be added to cell culture medium to inhibit the effects of the Zcytor21 ligand produced by the cultured cells.

Fusion proteins of Zcytor21 can be used to express *Zcytor21* in a recombinant host, and to isolate the produced Zcytor21. As described below, particular Zcytor21 fusion proteins also have uses in diagnosis and therapy. One type of fusion protein comprises a peptide that guides a Zcytor21 polypeptide from a recombinant host cell. To direct a Zcytor21 polypeptide into the secretory pathway of a eukaryotic host cell, a secretory signal sequence (also known as a signal peptide, a leader sequence, prepro sequence or pre sequence) is provided in the *Zcytor21* expression vector. While the secretory signal sequence may be derived from Zcytor21, a suitable signal sequence

may also be derived from another secreted protein or synthesized *de novo*. The secretory signal sequence is operably linked to a *Zcytor21*-encoding sequence such that the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the nucleotide sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the nucleotide sequence of interest (see, *e.g.*, Welch *et al.*, U.S. Patent No. 5,037,743; Holland *et al.*, U.S. Patent No. 5,143,830).

Although the secretory signal sequence of Zcytor21 or another protein produced by mammalian cells (*e.g.*, tissue-type plasminogen activator signal sequence, as described, for example, in U.S. Patent No. 5,641,655) is useful for expression of Zcytor21 in recombinant mammalian hosts, a yeast signal sequence is preferred for expression in yeast cells. Examples of suitable yeast signal sequences are those derived from yeast mating phermone α-factor (encoded by the *MFα1* gene), invertase (encoded by the *SUC2* gene), or acid phosphatase (encoded by the *PHO5* gene). See, for example, Romanos *et al.*, "Expression of Cloned Genes in Yeast," in *DNA Cloning 2: A Practical Approach,* 2nd Edition, Glover and Hames (eds.), pages 123-167 (Oxford University Press 1995).

In bacterial cells, it is often desirable to express a heterologous protein as a fusion protein to decrease toxicity, increase stability, and to enhance recovery of the expressed protein. For example, *Zcytor21* can be expressed as a fusion protein comprising a glutathione S-transferase polypeptide. Glutathione S-transferease fusion proteins are typically soluble, and easily purifiable from *E. coli* lysates on immobilized glutathione columns. In similar approaches, a Zcytor21 fusion protein comprising a maltose binding protein polypeptide can be isolated with an amylose resin column, while a fusion protein comprising the C-terminal end of a truncated Protein A gene can be purified using IgG-Sepharose. Established techniques for expressing a heterologous polypeptide as a fusion protein in a bacterial cell are described, for example, by Williams *et al.*, "Expression of Foreign Proteins in *E. coli* Using Plasmid Vectors and Purification of Specific Polyclonal Antibodies," in *DNA Cloning 2: A Practical Approach,* 2nd Edition, Glover and Hames (Eds.), pages 15-58 (Oxford University Press

1995). In addition, commercially available expression systems are available. For example, the PINPOINT Xa protein purification system (Promega Corporation; Madison, WI) provides a method for isolating a fusion protein comprising a polypeptide that becomes biotinylated during expression with a resin that comprises avidin.

Peptide tags that are useful for isolating heterologous polypeptides expressed by either prokaryotic or eukaryotic cells include polyHistidine tags (which have an affinity for nickel-chelating resin), *c-myc* tags, calmodulin binding protein (isolated with calmodulin affinity chromatography), substance P, the RYIRS tag (which binds with anti-RYIRS antibodies), the Glu-Glu tag, and the FLAG tag (which binds with anti-FLAG antibodies). See, for example, Luo *et al., Arch. Biochem. Biophys. 329*:215 (1996), Morganti *et al., Biotechnol. Appl. Biochem. 23*:67 (1996), and Zheng *et al., Gene 186*:55 (1997). Nucleic acid molecules encoding such peptide tags are available, for example, from Sigma-Aldrich Corporation (St. Louis, MO).

The present invention also contemplates that the use of the secretory signal sequence contained in the Zcytor21 polypeptides of the present invention to direct other polypeptides into the secretory pathway. A signal fusion polypeptide can be made wherein a secretory signal sequence derived from, for example, amino acid residues 1 to 23 of SEQ ID NO:2 is operably linked to another polypeptide using methods known in the art and disclosed herein. The secretory signal sequence contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory pathway. Such constructs have numerous applications known in the art. For example, these novel secretory signal sequence fusion constructs can direct the secretion of an active component of a normally non-secreted protein, such as a receptor. Such fusions may be used in a transgenic animal or in a cultured recombinant host to direct peptides through the secretory pathway. With regard to the latter, exemplary polypeptides include pharmaceutically active molecules such as Factor VIIa, proinsulin, insulin, follicle stimulating hormone, tissue type plasminogen activator, tumor necrosis factor, interleukins (*e.g.*, interleukin-1 (IL-1), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21), colony stimulating factors (*e.g.*, granulocyte-colony stimulating factor (G-CSF) and

granulocyte macrophage-colony stimulating factor (GM-CSF)), interferons (*e.g.*, interferons-α, -β, -γ, -ω, -δ, -ε, and -τ), the stem cell growth factor designated "S1 factor," erythropoietin, and thrombopoietin. The Zcytor21 secretory signal sequence contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory pathway. Fusion proteins comprising a Zcytor21 secretory signal sequence can be constructed using standard techniques.

Another form of fusion protein comprises a Zcytor21 polypeptide and an immunoglobulin heavy chain constant region, typically an F_C fragment, which contains two or three constant region domains and a hinge region but lacks the variable region. As an illustration, Chang *et al.*, U.S. Patent No. 5,723,125, describe a fusion protein comprising a human interferon and a human immunoglobulin Fc fragment. The C-terminal of the interferon is linked to the N-terminal of the Fc fragment by a peptide linker moiety. An example of a peptide linker is a peptide comprising primarily a T cell inert sequence, which is immunologically inert. An exemplary peptide linker has the amino acid sequence: GGSGG SGGGG SGGGG S (SEQ ID NO:13). In this fusion protein, an illustrative Fc moiety is a human γ4 chain, which is stable in solution and has little or no complement activating activity. Accordingly, the present invention contemplates a Zcytor21 fusion protein that comprises a Zcytor21 moiety and a human Fc fragment, wherein the C-terminus of the Zcytor21 moiety is attached to the N-terminus of the Fc fragment via a peptide linker, such as a peptide consisting of the amino acid sequence of SEQ ID NO:13. The Zcytor21 moiety can be a Zcytor21 molecule or a fragment thereof. For example, a fusion protein can comprise an Fc fragment (*e.g.*, a human Fc fragment), and amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444 of SEQ ID NO:15.

In another variation, a Zcytor21 fusion protein comprises an IgG sequence, a Zcytor21 moiety covalently joined to the amino terminal end of the IgG sequence, and a signal peptide that is covalently joined to the amino terminal of the Zcytor21 moiety, wherein the IgG sequence consists of the following elements in the

following order: a hinge region, a CH_2 domain, and a CH_3 domain. Accordingly, the IgG sequence lacks a CH_1 domain. The Zcytor21 moiety displays a Zcytor21 activity, as described herein, such as the ability to bind with a Zcytor21 ligand. This general approach to producing fusion proteins that comprise both antibody and nonantibody portions has been described by LaRochelle *et al.*, EP 742830 (WO 95/21258).

Fusion proteins comprising a Zcytor21 moiety and an Fc moiety can be used, for example, as an *in vitro* assay tool. For example, the presence of a Zcytor21 ligand in a biological sample can be detected using a Zcytor21-immunoglobulin fusion protein, in which the Zcytor21 moiety is used to bind the ligand, and a macromolecule, such as Protein A or anti-Fc antibody, is used to bind the fusion protein to a solid support. Such systems can be used to identify agonists and antagonists that interfere with the binding of a Zcytor21 ligand to its receptor.

Other examples of antibody fusion proteins include polypeptides that comprise an antigen-binding domain and a Zcytor21 fragment that contains a Zcytor21 extracellular domain. Such molecules can be used to target particular tissues for the benefit of Zcytor21 binding activity.

The present invention further provides a variety of other polypeptide fusions. For example, part or all of a domain(s) conferring a biological function can be swapped between Zcytor21 of the present invention with the functionally equivalent domain(s) from another member of the cytokine receptor family. Polypeptide fusions can be expressed in recombinant host cells to produce a variety of Zcytor21 fusion analogs. A Zcytor21 polypeptide can be fused to two or more moieties or domains, such as an affinity tag for purification and a targeting domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, for example, Tuan *et al., Connective Tissue Research 34*:1 (1996).

Fusion proteins can be prepared by methods known to those skilled in the art by preparing each component of the fusion protein and chemically conjugating them. Alternatively, a polynucleotide encoding both components of the fusion protein in the proper reading frame can be generated using known techniques and expressed by the methods described herein. General methods for enzymatic and chemical cleavage

of fusion proteins are described, for example, by Ausubel (1995) at pages 16-19 to 16-25.

Zcytor21 polypeptides can be used to identify and to isolate Zcytor21 ligands. For example, proteins and peptides of the present invention can be immobilized on a column and used to bind ligands from a biological sample that is run over the column (Hermanson *et al.* (eds.), *Immobilized Affinity Ligand Techniques*, pages 195-202 (Academic Press 1992)).

The activity of a Zcytor21 polypeptide can be observed by a silicon-based biosensor microphysiometer, which measures the extracellular acidification rate or proton excretion associated with receptor binding and subsequent physiologic cellular responses. An exemplary device is the CYTOSENSOR Microphysiometer manufactured by Molecular Devices, Sunnyvale, CA. A variety of cellular responses, such as cell proliferation, ion transport, energy production, inflammatory response, regulatory and receptor activation, and the like, can be measured by this method (see, for example, McConnell *et al., Science 257*:1906 (1992), Pitchford *et al., Meth. Enzymol. 228*:84 (1997), Arimilli *et al., J. Immunol. Meth. 212*:49 (1998), Van Liefde *et al., Eur. J. Pharmacol. 346*:87 (1998)). The microphysiometer can be used for assaying eukaryotic, prokaryotic, adherent, or non-adherent cells. By measuring extracellular acidification changes in cell media over time, the microphysiometer directly measures cellular responses to various stimuli, including agonists, ligands, or antagonists of Zcytor21.

For example, the microphysiometer is used to measure responses of an Zcytor21-expressing eukaryotic cell, compared to a control eukaryotic cell that does not express Zcytor21 polypeptide. Suitable cells responsive to Zcytor21-modulating stimuli include recombinant host cells comprising a Zcytor21 expression vector, and cells that naturally express Zcytor21. Extracellular acidification provides one measure for a Zcytor21-modulated cellular response. In addition, this approach can be used to identify ligands, agonists, and antagonists of Zcytor21 ligand. For example, a molecule can be identified as an agonist of Zcytor21 ligand by providing cells that express a Zcytor21 polypeptide, culturing a first portion of the cells in the absence of the test compound, culturing a second portion of the cells in the presence of the test compound,

and determining whether the second portion exhibits a cellular response, in comparison with the first portion.

Alternatively, a solid phase system can be used to identify a Zcytor21 ligand, or an agonist or antagonist of a Zcytor21 ligand. For example, a Zcytor21 polypeptide or Zcytor21 fusion protein can be immobilized onto the surface of a receptor chip of a commercially available biosensor instrument (BIACORE, Biacore AB; Uppsala, Sweden). The use of this instrument is disclosed, for example, by Karlsson, *Immunol. Methods 145*:229 (1991), and Cunningham and Wells, *J. Mol. Biol. 234*:554 (1993).

In brief, a Zcytor21 polypeptide or fusion protein is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within a flow cell. A test sample is then passed through the cell. If a ligand is present in the sample, it will bind to the immobilized polypeptide or fusion protein, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding. This system can also be used to examine antibody-antigen interactions, and the interactions of other complement/anti-complement pairs.

Zcytor21 binding domains can be further characterized by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids of Zcytor21 ligand agonists. See, for example, de Vos *et al., Science 255*:306 (1992), Smith *et al., J. Mol. Biol. 224*:899 (1992), and Wlodaver *et al., FEBS Lett. 309*:59 (1992).

The present invention also contemplates chemically modified Zcytor21 compositions, in which a Zcytor21 polypeptide is linked with a polymer. Illustrative Zcytor21 polypeptides are soluble polypeptides that lack a functional transmembrane domain, such as a polypeptide consisting of amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444 of SEQ ID NO:15. Typically, the polymer is water-soluble so that

the Zcytor21 conjugate does not precipitate in an aqueous environment, such as a physiological environment. An example of a suitable polymer is one that has been modified to have a single reactive group, such as an active ester for acylation, or an aldehyde for alkylation. In this way, the degree of polymerization can be controlled. An example of a reactive aldehyde is polyethylene glycol propionaldehyde, or mono-(C_1-C_{10}) alkoxy, or aryloxy derivatives thereof (see, for example, Harris, *et al.*, U.S. Patent No. 5,252,714). The polymer may be branched or unbranched. Moreover, a mixture of polymers can be used to produce Zcytor21 conjugates.

Zcytor21 conjugates used for therapy can comprise pharmaceutically acceptable water-soluble polymer moieties. Suitable water-soluble polymers include polyethylene glycol (PEG), monomethoxy-PEG, mono-(C_1-C_{10})alkoxy-PEG, aryloxy-PEG, poly-(N-vinyl pyrrolidone)PEG, tresyl monomethoxy PEG, PEG propionaldehyde, *bis*-succinimidyl carbonate PEG, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, dextran, cellulose, or other carbohydrate-based polymers. Suitable PEG may have a molecular weight from about 600 to about 60,000, including, for example, 5,000, 12,000, 20,000 and 25,000. A Zcytor21 conjugate can also comprise a mixture of such water-soluble polymers.

One example of a Zcytor21 conjugate comprises a Zcytor21 moiety and a polyalkyl oxide moiety attached to the *N*-terminus of the Zcytor21 moiety. PEG is one suitable polyalkyl oxide. As an illustration, Zcytor21 can be modified with PEG, a process known as "PEGylation." PEGylation of Zcytor21 can be carried out by any of the PEGylation reactions known in the art (see, for example, EP 0 154 316, Delgado *et al., Critical Reviews in Therapeutic Drug Carrier Systems 9*:249 (1992), Duncan and Spreafico, *Clin. Pharmacokinet. 27*:290 (1994), and Francis *et al., Int J Hematol 68*:1 (1998)). For example, PEGylation can be performed by an acylation reaction or by an alkylation reaction with a reactive polyethylene glycol molecule. In an alternative approach, Zcytor21 conjugates are formed by condensing activated PEG, in which a terminal hydroxy or amino group of PEG has been replaced by an activated linker (see, for example, Karasiewicz *et al.*, U.S. Patent No. 5,382,657).

PEGylation by acylation typically requires reacting an active ester derivative of PEG with a Zcytor21 polypeptide. An example of an activated PEG ester is PEG esterified to *N*-hydroxysuccinimide. As used herein, the term "acylation" includes the following types of linkages between Zcytor21 and a water-soluble polymer: amide, carbamate, urethane, and the like. Methods for preparing PEGylated Zcytor21 by acylation will typically comprise the steps of (a) reacting a Zcytor21 polypeptide with PEG (such as a reactive ester of an aldehyde derivative of PEG) under conditions whereby one or more PEG groups attach to Zcytor21, and (b) obtaining the reaction product(s). Generally, the optimal reaction conditions for acylation reactions will be determined based upon known parameters and desired results. For example, the larger the ratio of PEG:Zcytor21, the greater the percentage of polyPEGylated Zcytor21 product.

The product of PEGylation by acylation is typically a polyPEGylated Zcytor21 product, wherein the lysine ε-amino groups are PEGylated via an acyl linking group. An example of a connecting linkage is an amide. Typically, the resulting Zcytor21 will be at least 95% mono-, di-, or tri-pegylated, although some species with higher degrees of PEGylation may be formed depending upon the reaction conditions. PEGylated species can be separated from unconjugated Zcytor21 polypeptides using standard purification methods, such as dialysis, ultrafiltration, ion exchange chromatography, affinity chromatography, and the like.

PEGylation by alkylation generally involves reacting a terminal aldehyde derivative of PEG with Zcytor21 in the presence of a reducing agent. PEG groups can be attached to the polypeptide via a -CH_2-NH group.

Derivatization via reductive alkylation to produce a monoPEGylated product takes advantage of the differential reactivity of different types of primary amino groups available for derivatization. Typically, the reaction is performed at a pH that allows one to take advantage of the pKa differences between the ε-amino groups of the lysine residues and the α-amino group of the *N*-terminal residue of the protein. By such selective derivatization, attachment of a water-soluble polymer that contains a reactive group such as an aldehyde, to a protein is controlled. The conjugation with the polymer occurs predominantly at the *N*-terminus of the protein without significant modification

of other reactive groups such as the lysine side chain amino groups. The present invention provides a substantially homogenous preparation of Zcytor21 monopolymer conjugates.

Reductive alkylation to produce a substantially homogenous population of monopolymer Zcytor21 conjugate molecule can comprise the steps of: (a) reacting a Zcytor21 polypeptide with a reactive PEG under reductive alkylation conditions at a pH suitable to permit selective modification of the α-amino group at the amino terminus of the Zcytor21, and (b) obtaining the reaction product(s). The reducing agent used for reductive alkylation should be stable in aqueous solution and able to reduce only the Schiff base formed in the initial process of reductive alkylation. Illustrative reducing agents include sodium borohydride, sodium cyanoborohydride, dimethylamine borane, trimethylamine borane, and pyridine borane.

For a substantially homogenous population of monopolymer Zcytor21 conjugates, the reductive alkylation reaction conditions are those that permit the selective attachment of the water soluble polymer moiety to the *N*-terminus of Zcytor21. Such reaction conditions generally provide for pKa differences between the lysine amino groups and the α-amino group at the *N*-terminus. The pH also affects the ratio of polymer to protein to be used. In general, if the pH is lower, a larger excess of polymer to protein will be desired because the less reactive the *N*-terminal α-group, the more polymer is needed to achieve optimal conditions. If the pH is higher, the polymer:Zcytor21 need not be as large because more reactive groups are available. Typically, the pH will fall within the range of 3 to 9, or 3 to 6.

Another factor to consider is the molecular weight of the water-soluble polymer. Generally, the higher the molecular weight of the polymer, the fewer number of polymer molecules which may be attached to the protein. For PEGylation reactions, the typical molecular weight is about 2 kDa to about 100 kDa, about 5 kDa to about 50 kDa, or about 12 kDa to about 25 kDa. The molar ratio of water-soluble polymer to Zcytor21 will generally be in the range of 1:1 to 100:1. Typically, the molar ratio of water-soluble polymer to Zcytor21 will be 1:1 to 20:1 for polyPEGylation, and 1:1 to 5:1 for monoPEGylation.

General methods for producing conjugates comprising a polypeptide and water-soluble polymer moieties are known in the art. See, for example, Karasiewicz *et al.*, U.S. Patent No. 5,382,657, Greenwald *et al.*, U.S. Patent No. 5,738, 846, Nieforth *et al.*, *Clin. Pharmacol. Ther. 59*:636 (1996), Monkarsh *et al.*, *Anal. Biochem. 247*:434 (1997)).

The present invention contemplates compositions comprising a peptide or polypeptide described herein. Such compositions can further comprise a carrier. The carrier can be a conventional organic or inorganic carrier. Examples of carriers include water, buffer solution, alcohol, propylene glycol, macrogol, sesame oil, corn oil, and the like.

7. *Isolation of Zcytor21 Polypeptides*

The polypeptides of the present invention can be purified to at least about 80% purity, to at least about 90% purity, to at least about 95% purity, or greater than 95% purity with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. The polypeptides of the present invention may also be purified to a pharmaceutically pure state, which is greater than 99.9% pure. In certain preparations, purified polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin.

Fractionation and/or conventional purification methods can be used to obtain preparations of Zcytor21 purified from natural sources (*e.g.*, skin tissue), synthetic Zcytor21 polypeptides, and recombinant Zcytor21 polypeptides and fusion Zcytor21 polypeptides purified from recombinant host cells. In general, ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable chromatographic media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are suitable. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the

like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties.

Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Selection of a particular method for polypeptide isolation and purification is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, *Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology 1988), and Doonan, *Protein Purification Protocols* (The Humana Press 1996).

Additional variations in Zcytor21 isolation and purification can be devised by those of skill in the art. For example, anti-Zcytor21 antibodies, obtained as described below, can be used to isolate large quantities of protein by immunoaffinity purification.

The polypeptides of the present invention can also be isolated by exploitation of particular properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins, including those comprising polyhistidine tags. Briefly, a gel is first charged with divalent metal ions to form a chelate (Sulkowski, *Trends in Biochem.* *3*:1 (1985)). Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography (M. Deutscher, (ed.), *Meth. Enzymol.* *182*:529 (1990)). Within additional embodiments of the invention, a

fusion of the polypeptide of interest and an affinity tag (*e.g.*, maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification.

Zcytor21 polypeptides or fragments thereof may also be prepared through chemical synthesis, as described above. Zcytor21 polypeptides may be monomers or multimers; glycosylated or non-glycosylated; PEGylated or non-PEGylated; and may or may not include an initial methionine amino acid residue.

8. *Production of Antibodies to Zcytor21 Proteins*

Antibodies to Zcytor21 can be obtained, for example, using the product of a Zcytor21 expression vector or Zcytor21 isolated from a natural source as an antigen. Particularly useful anti-Zcytor21 antibodies "bind specifically" with Zcytor21. Antibodies are considered to be specifically binding if the antibodies exhibit at least one of the following two properties: (1) antibodies bind to Zcytor21 with a threshold level of binding activity, and (2) antibodies do not significantly cross-react with polypeptides related to Zcytor21.

With regard to the first characteristic, antibodies specifically bind if they bind to a Zcytor21 polypeptide, peptide or epitope with a binding affinity (K_a) of 10^6 M^{-1} or greater, preferably 10^7 M^{-1} or greater, more preferably 10^8 M^{-1} or greater, and most preferably 10^9 M^{-1} or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, *Ann. NY Acad. Sci. 51*:660 (1949)). With regard to the second characteristic, antibodies do not significantly cross-react with related polypeptide molecules, for example, if they detect Zcytor21, but not presently known polypeptides using a standard Western blot analysis. Examples of known related polypeptides include known cytokine receptors.

Anti-Zcytor21 antibodies can be produced using antigenic Zcytor21 epitope-bearing peptides and polypeptides. Antigenic epitope-bearing peptides and polypeptides of the present invention contain a sequence of at least nine, or between 15 to about 30 amino acids contained within SEQ ID NO:2 or another amino acid sequence disclosed herein. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of the invention, containing from 30 to 50 amino

acids, or any length up to and including the entire amino acid sequence of a polypeptide of the invention, also are useful for inducing antibodies that bind with Zcytor21. It is desirable that the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (*i.e.*, the sequence includes relatively hydrophilic residues, while hydrophobic residues are typically avoided). Moreover, amino acid sequences containing proline residues may be also be desirable for antibody production.

As an illustration, potential antigenic sites in Zcytor21 were identified using the Jameson-Wolf method, Jameson and Wolf, *CABIOS 4*:181, (1988), as implemented by the PROTEAN program (version 3.14) of LASERGENE (DNASTAR; Madison, WI). Default parameters were used in this analysis.

The Jameson-Wolf method predicts potential antigenic determinants by combining six major subroutines for protein structural prediction. Briefly, the Hopp-Woods method, Hopp *et al., Proc. Nat'l Acad. Sci. USA 78*:3824 (1981), was first used to identify amino acid sequences representing areas of greatest local hydrophilicity (parameter: seven residues averaged). In the second step, Emini's method, Emini *et al., J. Virology 55*:836 (1985), was used to calculate surface probabilities (parameter: surface decision threshold (0.6) = 1). Third, the Karplus-Schultz method, Karplus and Schultz, *Naturwissenschaften 72*:212 (1985), was used to predict backbone chain flexibility (parameter: flexibility threshold (0.2) = 1). In the fourth and fifth steps of the analysis, secondary structure predictions were applied to the data using the methods of Chou-Fasman, Chou, "Prediction of Protein Structural Classes from Amino Acid Composition," in *Prediction of Protein Structure and the Principles of Protein Conformation*, Fasman (ed.), pages 549-586 (Plenum Press 1990), and Garnier-Robson, Garnier *et al., J. Mol. Biol. 120*:97 (1978) (Chou-Fasman parameters: conformation table = 64 proteins; α region threshold = 103; β region threshold = 105; Garnier-Robson parameters: α and β decision constants = 0). In the sixth subroutine, flexibility parameters and hydropathy/solvent accessibility factors were combined to determine a surface contour value, designated as the "antigenic index." Finally, a peak broadening function was applied to the antigenic index, which broadens major surface peaks by adding 20, 40, 60, or 80% of the respective peak value to account for additional free

energy derived from the mobility of surface regions relative to interior regions. This calculation was not applied, however, to any major peak that resides in a helical region, since helical regions tend to be less flexible.

The results of this analysis indicated that the following amino acid sequences of SEQ ID NO:11 would provide suitable antigenic molecules: amino acids 44 to 52, amino acids 101 to 109, amino acids 121 to 136, amino acids 141 to 180, amino acids 250 to 261, amino acids 313 to 328, amino acids 477 to 488, amino acids 562 to 574, amino acids 597 to 622, and amino acids 641 to 652. The present invention contemplates the use of any one of these antigenic amino acid sequences to generate antibodies to Zcytor21-d2. The present invention also contemplates polypeptides comprising at least one of these antigenic molecules. Similar analyses can be performed with the other Zcytor21 amino acid sequences disclosed herein.

Polyclonal antibodies to recombinant Zcytor21 protein or to Zcytor21 isolated from natural sources can be prepared using methods well-known to those of skill in the art. See, for example, Green *et al.*, "Production of Polyclonal Antisera," in *Immunochemical Protocols* (Manson, ed.), pages 1-5 (Humana Press 1992), and Williams *et al.*, "Expression of foreign proteins in *E. coli* using plasmid vectors and purification of specific polyclonal antibodies," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), page 15 (Oxford University Press 1995). The immunogenicity of a Zcytor21 polypeptide can be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of Zcytor21 or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like," such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

Although polyclonal antibodies are typically raised in animals such as horses, cows, dogs, chicken, rats, mice, rabbits, guinea pigs, goats, or sheep, an anti-Zcytor21 antibody of the present invention may also be derived from a subhuman primate antibody. General techniques for raising diagnostically and therapeutically

useful antibodies in baboons may be found, for example, in Goldenberg *et al.*, international patent publication No. WO 91/11465, and in Losman *et al.*, *Int. J. Cancer* *46*:310 (1990).

Alternatively, monoclonal anti-Zcytor21 antibodies can be generated. Rodent monoclonal antibodies to specific antigens may be obtained by methods known to those skilled in the art (see, for example, Kohler *et al.*, *Nature 256*:495 (1975), Coligan *et al.* (eds.), *Current Protocols in Immunology, Vol. 1*, pages 2.5.1-2.6.7 (John Wiley & Sons 1991) ["Coligan"], Picksley *et al.*, "Production of monoclonal antibodies against proteins expressed in *E. coli*," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), page 93 (Oxford University Press 1995)).

Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising a *Zcytor21* gene product, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B-lymphocytes, fusing the B-lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones which produce antibodies to the antigen, culturing the clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures.

In addition, an anti-Zcytor21 antibody of the present invention may be derived from a human monoclonal antibody. Human monoclonal antibodies are obtained from transgenic mice that have been engineered to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described, for example, by Green *et al.*, *Nature Genet. 7*:13 (1994), Lonberg *et al.*, *Nature 368*:856 (1994), and Taylor *et al.*, *Int. Immun. 6*:579 (1994).

Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and

ion-exchange chromatography (see, for example, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3; Baines *et al.,* "Purification of Immunoglobulin G (IgG)," in *Methods in Molecular Biology, Vol. 10,* pages 79-104 (The Humana Press, Inc. 1992)).

For particular uses, it may be desirable to prepare fragments of anti-Zcytor21 antibodies. Such antibody fragments can be obtained, for example, by proteolytic hydrolysis of the antibody. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. As an illustration, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent to produce 3.5S Fab' monovalent fragments. Optionally, the cleavage reaction can be performed using a blocking group for the sulfhydryl groups that result from cleavage of disulfide linkages. As an alternative, an enzymatic cleavage using pepsin produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. patent No. 4,331,647, Nisonoff *et al., Arch Biochem. Biophys. 89*:230 (1960), Porter, *Biochem. J. 73*:119 (1959), Edelman *et al.*, in *Methods in Enzymology Vol. 1*, page 422 (Academic Press 1967), and by Coligan at pages 2.8.1-2.8.10 and 2.10.-2.10.4.

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

For example, Fv fragments comprise an association of V_H and V_L chains. This association can be noncovalent, as described by Inbar *et al., Proc. Nat'l Acad. Sci. USA 69*:2659 (1972). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde (see, for example, Sandhu, *Crit. Rev. Biotech. 12*:437 (1992)).

The Fv fragments may comprise V_H and V_L chains, which are connected by a peptide linker. These single-chain antigen binding proteins (scFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains which are connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell, such as *E.*

coli. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing scFvs are described, for example, by Whitlow *et al., Methods: A Companion to Methods in Enzymology* 2:97 (1991) (also see, Bird *et al., Science 242*:423 (1988), Ladner *et al.*, U.S. Patent No. 4,946,778, Pack *et al., Bio/Technology 11*:1271 (1993), and Sandhu, *supra*).

As an illustration, a scFV can be obtained by exposing lymphocytes to Zcytor21 polypeptide *in vitro*, and selecting antibody display libraries in phage or similar vectors (for instance, through use of immobilized or labeled Zcytor21 protein or peptide). Genes encoding polypeptides having potential Zcytor21 polypeptide binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides, which interact with a known target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such random peptide display libraries are known in the art (Ladner *et al.*, U.S. Patent No. 5,223,409, Ladner *et al.*, U.S. Patent No. 4,946,778, Ladner *et al.*, U.S. Patent No. 5,403,484, Ladner *et al.*, U.S. Patent No. 5,571,698, and Kay *et al., Phage Display of Peptides and Proteins* (Academic Press, Inc. 1996)) and random peptide display libraries and kits for screening such libraries are available commercially, for instance from CLONTECH Laboratories, Inc. (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA), and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the Zcytor21 sequences disclosed herein to identify proteins which bind to Zcytor21.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells (see, for example, Larrick *et al., Methods: A Companion to Methods in Enzymology* 2:106

(1991), Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in *Monoclonal Antibodies: Production, Engineering and Clinical Application*, Ritter *et al.* (eds.), page 166 (Cambridge University Press 1995), and Ward *et al.*, "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, Birch *et al.*, (eds.), page 137 (Wiley-Liss, Inc. 1995)).

Alternatively, an anti-Zcytor21 antibody may be derived from a "humanized" monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementary determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain. Typical residues of human antibodies are then substituted in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi *et al., Proc. Nat'l Acad. Sci. USA 86*:3833 (1989). Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al., Nature 321*:522 (1986), Carter *et al., Proc. Nat'l Acad. Sci. USA 89*:4285 (1992), Sandhu, *Crit. Rev. Biotech. 12*:437 (1992), Singer *et al., J. Immun. 150*:2844 (1993), Sudhir (ed.), *Antibody Engineering Protocols* (Humana Press, Inc. 1995), Kelley, "Engineering Therapeutic Antibodies," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 399-434 (John Wiley & Sons, Inc. 1996), and by Queen *et al.*, U.S. Patent No. 5,693,762 (1997).

Polyclonal anti-idiotype antibodies can be prepared by immunizing animals with anti-Zcytor21 antibodies or antibody fragments, using standard techniques. See, for example, Green *et al.*, "Production of Polyclonal Antisera," in *Methods In Molecular Biology: Immunochemical Protocols*, Manson (ed.), pages 1-12 (Humana Press 1992). Also, see Coligan at pages 2.4.1-2.4.7. Alternatively, monoclonal anti-idiotype antibodies can be prepared using anti-Zcytor21 antibodies or antibody fragments as immunogens with the techniques, described above. As another alternative, humanized anti-idiotype antibodies or subhuman primate anti-idiotype antibodies can be prepared using the above-described techniques. Methods for producing anti-idiotype antibodies are described, for example, by Irie, U.S. Patent No.

5,208,146, Greene, *et. al.*, U.S. Patent No. 5,637,677, and Varthakavi and Minocha, *J. Gen. Virol.* *77*:1875 (1996).

9. *Use of Zcytor21 Nucleotide Sequences to Detect Gene Expression and Gene Structure*

Nucleic acid molecules can be used to detect the expression of a *Zcytor21* gene in a biological sample. Suitable probe molecules include double-stranded nucleic acid molecules comprising the nucleotide sequence of SEQ ID NOs:1, 4, 7, 10, or 14, or a portion thereof, as well as single-stranded nucleic acid molecules having the complement of the nucleotide sequence of SEQ ID NOs:1, 4, 7, 10, or 14, or a portion thereof. Probe molecules may be DNA, RNA, oligonucleotides, and the like. As used herein, the term "portion" refers to at least eight nucleotides to at least 20 or more nucleotides. Illustrative probes bind with regions of the *Zcytor21* gene that have a low sequence similarity to comparable regions in other cytokine receptor genes.

In a basic assay, a single-stranded probe molecule is incubated with RNA, isolated from a biological sample, under conditions of temperature and ionic strength that promote base pairing between the probe and target *Zcytor21* RNA species. After separating unbound probe from hybridized molecules, the amount of hybrids is detected.

Well-established hybridization methods of RNA detection include northern analysis and dot/slot blot hybridization (see, for example, Ausubel (1995) at pages 4-1 to 4-27, and Wu *et al.* (eds.), "Analysis of Gene Expression at the RNA Level," in *Methods in Gene Biotechnology*, pages 225-239 (CRC Press, Inc. 1997)). Nucleic acid probes can be detectably labeled with radioisotopes such as ^{32}P or ^{35}S. Alternatively, Zcytor21 RNA can be detected with a nonradioactive hybridization method (see, for example, Isaac (ed.), *Protocols for Nucleic Acid Analysis by Nonradioactive Probes* (Humana Press, Inc. 1993)). Typically, nonradioactive detection is achieved by enzymatic conversion of chromogenic or chemiluminescent substrates. Illustrative nonradioactive moieties include biotin, fluorescein, and digoxigenin.

Zcytor21 oligonucleotide probes are also useful for *in vivo* diagnosis. As an illustration, ^{18}F-labeled oligonucleotides can be administered to a subject and

visualized by positron emission tomography (Tavitian *et al., Nature Medicine 4*:467 (1998)).

Numerous diagnostic procedures take advantage of the polymerase chain reaction (PCR) to increase sensitivity of detection methods. Standard techniques for performing PCR are well-known (see, generally, Mathew (ed.), *Protocols in Human Molecular Genetics* (Humana Press, Inc. 1991), White (ed.), *PCR Protocols: Current Methods and Applications* (Humana Press, Inc. 1993), Cotter (ed.), *Molecular Diagnosis of Cancer* (Humana Press, Inc. 1996), Hanausek and Walaszek (eds.), *Tumor Marker Protocols* (Humana Press, Inc. 1998), Lo (ed.), *Clinical Applications of PCR* (Humana Press, Inc. 1998), and Meltzer (ed.), *PCR in Bioanalysis* (Humana Press, Inc. 1998)).

PCR primers can be designed to amplify a portion of the *Zcytor21* gene that has a low sequence similarity to a comparable region in other proteins, such as other cytokine receptor proteins.

One variation of PCR for diagnostic assays is reverse transcriptase-PCR (RT-PCR). In the RT-PCR technique, RNA is isolated from a biological sample, reverse transcribed to cDNA, and the cDNA is incubated with *Zcytor21* primers (see, for example, Wu *et al.* (eds.), "Rapid Isolation of Specific cDNAs or Genes by PCR," in *Methods in Gene Biotechnology*, pages 15-28 (CRC Press, Inc. 1997)). PCR is then performed and the products are analyzed using standard techniques.

As an illustration, RNA is isolated from biological sample using, for example, the guanidinium-thiocyanate cell lysis procedure described above. Alternatively, a solid-phase technique can be used to isolate mRNA from a cell lysate. A reverse transcription reaction can be primed with the isolated RNA using random oligonucleotides, short homopolymers of dT, or *Zcytor21* anti-sense oligomers. Oligo-dT primers offer the advantage that various mRNA nucleotide sequences are amplified that can provide control target sequences. Zcytor21 sequences are amplified by the polymerase chain reaction using two flanking oligonucleotide primers that are typically 20 bases in length.

PCR amplification products can be detected using a variety of approaches. For example, PCR products can be fractionated by gel electrophoresis, and

visualized by ethidium bromide staining. Alternatively, fractionated PCR products can be transferred to a membrane, hybridized with a detectably-labeled Zcytor21 probe, and examined by autoradiography. Additional alternative approaches include the use of digoxigenin-labeled deoxyribonucleic acid triphosphates to provide chemiluminescence detection, and the C-TRAK colorimetric assay.

Another approach for detection of *Zcytor21* expression is cycling probe technology, in which a single-stranded DNA target binds with an excess of DNA-RNA-DNA chimeric probe to form a complex, the RNA portion is cleaved with RNAase H, and the presence of cleaved chimeric probe is detected (see, for example, Beggs *et al.*, *J. Clin. Microbiol.* *34*:2985 (1996), Bekkaoui *et al.*, *Biotechniques* *20*:240 (1996)). Alternative methods for detection of *Zcytor21* sequences can utilize approaches such as nucleic acid sequence-based amplification, cooperative amplification of templates by cross-hybridization, and the ligase chain reaction (see, for example, Marshall *et al.*, U.S. Patent No. 5,686,272 (1997), Dyer *et al.*, *J. Virol. Methods* *60*:161 (1996), Ehricht *et al.*, *Eur. J. Biochem.* *243*:358 (1997), and Chadwick *et al.*, *J. Virol. Methods* *70*:59 (1998)). Other standard methods are known to those of skill in the art.

Zcytor21 probes and primers can also be used to detect and to localize *Zcytor21* gene expression in tissue samples. Methods for such *in situ* hybridization are well-known to those of skill in the art (see, for example, Choo (ed.), *In Situ Hybridization Protocols* (Humana Press, Inc. 1994), Wu *et al.* (eds.), "Analysis of Cellular DNA or Abundance of mRNA by Radioactive *In Situ* Hybridization (RISH)," in *Methods in Gene Biotechnology*, pages 259-278 (CRC Press, Inc. 1997), and Wu *et al.* (eds.), "Localization of DNA or Abundance of mRNA by Fluorescence *In Situ* Hybridization (RISH)," in *Methods in Gene Biotechnology*, pages 279-289 (CRC Press, Inc. 1997)). Various additional diagnostic approaches are well-known to those of skill in the art (see, for example, Mathew (ed.), *Protocols in Human Molecular Genetics* (Humana Press, Inc. 1991), Coleman and Tsongalis, *Molecular Diagnostics* (Humana Press, Inc. 1996), and Elles, *Molecular Diagnosis of Genetic Diseases* (Humana Press, Inc., 1996)). Suitable test samples include blood, urine, saliva, tissue biopsy, and autopsy material.

Mutations of cytokine receptors are associated with particular diseases. For example, polymorphisms of cytokine receptors are associated with pulmonary alveolar proteinosis, familial periodic fever, and erythroleukemia. The *Zcytor21* gene is located in human chromosome 3p25.3. This region is associated with various disorders and diseases, including Fanconi anemia, xeroderma pigmentosum, a Marfan-like connective tissue disorder, and cardiomyopathy. Thus, *Zcytor21* nucleotide sequences can be used in linkage-based testing for various diseases, and to determine whether a subject's chromosomes contain a mutation in the *Zcytor21* gene. Detectable chromosomal aberrations at the *Zcytor21* gene locus include, but are not limited to, aneuploidy, gene copy number changes, insertions, deletions, restriction site changes and rearrangements. Of particular interest are genetic alterations that inactivate a *Zcytor21* gene.

The present invention also provides reagents which will find use in diagnostic applications. For example, the zcytor21 gene, a probe comprising zcytor21 DNA or RNA or a subsequence thereof can be used to determine if the zcytor21 gene is present on a human chromosome, such as chromosome 3, or if a gene mutation has occurred. Based on annotation of a fragment of human genomic DNA containing a part of zcytor21 genomic DNA, zcytor21 is located at the p25.3 region of chromosome 3. Detectable chromosomal aberrations at the zcytor21 gene locus include, but are not limited to, aneuploidy, gene copy number changes, loss of heterozygosity (LOH), translocations, insertions, deletions, restriction site changes and rearrangements. Such aberrations can be detected using polynucleotides of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing PCR techniques, and other genetic linkage analysis techniques known in the art (Sambrook et al., *ibid.*; Ausubel et al., *ibid.*; Marian, *Chest 108*:255-65, 1995).

The precise knowledge of a gene's position can be useful for a number of purposes, including: 1) determining if a sequence is part of an existing contig and obtaining additional surrounding genetic sequences in various forms, such as YACs, BACs or cDNA clones; 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region; and 3) cross-referencing

model organisms, such as mouse, which may aid in determining what function a particular gene might have.

The zcytor21 gene is located at the p25.3 region of chromosome 3. Several genes of known function map to this region that are linked to human disease. Thus, since the zcytor21 gene maps to chromosome p25.3, the zcytor21 polynucleotide probes of the present invention can be used to detect and diagnose the presence of chromosome 3 monosomy and other chromosome p25.3 loss, and particularly chromosome 3 monosomy and loss and chromosomal aberrations at p25.3 including deletions, rearrangements, and chromosomal breakpoints, and translocations can be associated with tumors. Thus, since the zcytor21 gene maps to this critical region, the zcytor21 polynucleotide probes of the present invention can be used to detect chromosome deletions, translocations and rearrangements associated with those diseases. See the Online Mendellian Inheritance of Man (OMIM™, National Center for Biotechnology Information, National Library of Medicine. Bethesda, MD) gene map, and references therein, for this region of human chromosome 3, and p25.3 on a publicly available world wide web server. All of these serve as possible candidate genes for an inheritable disease that show linkage to the same chromosomal region as the zcytor21 gene. Thus, zcytor21 polynucleotide probes can be used to detect abnormalities or genotypes associated with these defects.

A diagnostic could assist physicians in determining the type of disease and appropriate associated therapy, or assistance in genetic counseling. As such, the inventive anti-zcytor21 antibodies, polynucleotides, and polypeptides can be used for the detection of zcytor21 polypeptide, mRNA or anti-zcytor21 antibodies, thus serving as markers and be directly used for detecting or genetic diseases or cancers, as described herein, using methods known in the art and described herein. Further, zcytor21 polynucleotide probes can be used to detect abnormalities or genotypes associated with chromosome p25.3 deletions, chromosome 3 monosomy and translocations associated with human diseases, such as described above, or other translocations and LOH involved with malignant progression of tumors or other p25.3 mutations, which are expected to be involved in chromosome rearrangements in malignancy; or in other cancers. Similarly, zcytor21 polynucleotide probes can be used

to detect abnormalities or genotypes associated with chromosome p25.3 trisomy and chromosome loss associated with human diseases or spontaneous abortion. All of these serve as possible candidate genes for an inheritable disease which show linkage to the same chromosomal region as the zcytor21 gene. Thus, zcytor21 polynucleotide probes can be used to detect abnormalities or genotypes associated with these defects.

One of skill in the art would recognize that of zcytor21 polynucleotide probes are particularly useful for diagnosis of gross chromosome 3 abnormalities associated with loss of heterogeneity (LOH), chromosome gain (e.g. trisomy), translocation, chromosome loss (monosomy), DNA amplification, and the like. Translocations within chromosomal locus p25.3 wherein the zcytor21 gene is located are known to be associated with human disease. For example, p25.3 deletions, monosomy and translocations are associated with specific human diseases as discussed above. Thus, since the zcytor21 gene maps to this critical region, zcytor21 polynucleotide probes of the present invention can be used to detect abnormalities or genotypes associated with p25.3 translocation, deletion and trisomy, and the like, described above.

As discussed above, defects in the zcytor21 gene itself may result in a heritable human disease state. Molecules of the present invention, such as the polypeptides, antagonists, agonists, polynucleotides and antibodies of the present invention would aid in the detection, diagnosis prevention, and treatment associated with a zcytor21 genetic defect. In addition, zcytor21 polynucleotide probes can be used to detect allelic differences between diseased or non-diseased individuals at the zcytor21 chromosomal locus. As such, the zcytor21 sequences can be used as diagnostics in forensic DNA profiling.

The protein truncation test is also useful for detecting the inactivation of a gene in which translation-terminating mutations produce only portions of the encoded protein (see, for example, Stoppa-Lyonnet *et al., Blood 91*:3920 (1998)). According to this approach, RNA is isolated from a biological sample, and used to synthesize cDNA. PCR is then used to amplify the *Zcytor21* target sequence and to introduce an RNA polymerase promoter, a translation initiation sequence, and an in-frame ATG triplet. PCR products are transcribed using an RNA polymerase, and the transcripts are

translated *in vitro* with a T7-coupled reticulocyte lysate system. The translation products are then fractionated by SDS-PAGE to determine the lengths of the translation products. The protein truncation test is described, for example, by Dracopoli *et al.* (eds.), *Current Protocols in Human Genetics,* pages 9.11.1 - 9.11.18 (John Wiley & Sons 1998).

The present invention also contemplates kits for performing a diagnostic assay for *Zcytor21* gene expression or to detect mutations in the *Zcytor21* gene. Such kits comprise nucleic acid probes, such as double-stranded nucleic acid molecules comprising the nucleotide sequence of nucleotides 135 to 1427 of SEQ ID NO:10, the nucleotide sequence of SEQ ID NO:10, or a portion thereof, as well as single-stranded nucleic acid molecules having the complement of the nucleotide sequence of SEQ ID NO:10, or a portion thereof. Nucleic acid probes can also be based upon the nucleotide sequences of SEQ ID NOs: 1, 4, 7, or 14. Probe molecules may be DNA, RNA, oligonucleotides, and the like. Kits may comprise nucleic acid primers for performing PCR.

Such kits can contain all the elements to perform a nucleic acid diagnostic assay described above. A kit will comprise at least one container comprising a *Zcytor21* probe or primer. The kit may also comprise a second container comprising one or more reagents capable of indicating the presence of *Zcytor21* sequences. Examples of such indicator reagents include detectable labels such as radioactive labels, fluorochromes, chemiluminescent agents, and the like. A kit may also comprise a means for conveying to the user that the *Zcytor21* probes and primers are used to detect *Zcytor21* gene expression. For example, written instructions may state that the enclosed nucleic acid molecules can be used to detect either a nucleic acid molecule that encodes Zcytor21, or a nucleic acid molecule having a nucleotide sequence that is complementary to a *Zcytor21*-encoding nucleotide sequence. The written material can be applied directly to a container, or the written material can be provided in the form of a packaging insert.

10. Use of Anti-Zcytor21 Antibodies to Detect Zcytor21

The present invention contemplates the use of anti-Zcytor21 antibodies to screen biological samples *in vitro* for the presence of Zcytor21. In one type of *in vitro* assay, anti-Zcytor21 antibodies are used in liquid phase. For example, the presence of Zcytor21 in a biological sample can be tested by mixing the biological sample with a trace amount of labeled Zcytor21 and an anti-Zcytor21 antibody under conditions that promote binding between Zcytor21 and its antibody. Complexes of Zcytor21 and anti-Zcytor21 in the sample can be separated from the reaction mixture by contacting the complex with an immobilized protein which binds with the antibody, such as an Fc antibody or *Staphylococcus* protein A. The concentration of Zcytor21 in the biological sample will be inversely proportional to the amount of labeled Zcytor21 bound to the antibody and directly related to the amount of free-labeled Zcytor21. Illustrative biological samples include blood, urine, saliva, tissue biopsy, and autopsy material.

Alternatively, *in vitro* assays can be performed in which anti-Zcytor21 antibody is bound to a solid-phase carrier. For example, antibody can be attached to a polymer, such as aminodextran, in order to link the antibody to an insoluble support such as a polymer-coated bead, a plate or a tube. Other suitable *in vitro* assays will be readily apparent to those of skill in the art.

In another approach, anti-Zcytor21 antibodies can be used to detect Zcytor21 in tissue sections prepared from a biopsy specimen. Such immunochemical detection can be used to determine the relative abundance of Zcytor21 and to determine the distribution of Zcytor21 in the examined tissue. General immunochemistry techniques are well established (see, for example, Ponder, "Cell Marking Techniques and Their Application," in *Mammalian Development: A Practical Approach*, Monk (ed.), pages 115-38 (IRL Press 1987), Coligan at pages 5.8.1-5.8.8, Ausubel (1995) at pages 14.6.1 to 14.6.13 (Wiley Interscience 1990), and Manson (ed.), *Methods In Molecular Biology, Vol. 10: Immunochemical Protocols* (The Humana Press, Inc. 1992)).

Immunochemical detection can be performed by contacting a biological sample with an anti-Zcytor21 antibody, and then contacting the biological sample with a detectably labeled molecule, which binds to the antibody. For example, the detectably labeled molecule can comprise an antibody moiety that binds to anti-Zcytor21 antibody.

Alternatively, the anti-Zcytor21 antibody can be conjugated with avidin/streptavidin (or biotin) and the detectably labeled molecule can comprise biotin (or avidin/streptavidin). Numerous variations of this basic technique are well-known to those of skill in the art.

Alternatively, an anti-Zcytor21 antibody can be conjugated with a detectable label to form an anti-Zcytor21 immunoconjugate. Suitable detectable labels include, for example, a radioisotope, a fluorescent label, a chemiluminescent label, an enzyme label, a bioluminescent label or colloidal gold. Methods of making and detecting such detectably-labeled immunoconjugates are well-known to those of ordinary skill in the art, and are described in more detail below.

The detectable label can be a radioisotope that is detected by autoradiography. Isotopes that are particularly useful for the purpose of the present invention are ^{3}H, ^{125}I, ^{131}I, ^{35}S and ^{14}C.

Anti-Zcytor21 immunoconjugates can also be labeled with a fluorescent compound. The presence of a fluorescently-labeled antibody is determined by exposing the immunoconjugate to light of the proper wavelength and detecting the resultant fluorescence. Fluorescent labeling compounds include fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

Alternatively, anti-Zcytor21 immunoconjugates can be detectably labeled by coupling an antibody component to a chemiluminescent compound. The presence of the chemiluminescent-tagged immunoconjugate is determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of chemiluminescent labeling compounds include luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester.

Similarly, a bioluminescent compound can be used to label anti-Zcytor21 immunoconjugates of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Bioluminescent compounds that are useful for labeling include luciferin, luciferase and aequorin.

Alternatively, anti-Zcytor21 immunoconjugates can be detectably labeled by linking an anti-Zcytor21 antibody component to an enzyme. When the anti-Zcytor21-

enzyme conjugate is incubated in the presence of the appropriate substrate, the enzyme moiety reacts with the substrate to produce a chemical moiety, which can be detected, for example, by spectrophotometric, fluorometric or visual means. Examples of enzymes that can be used to detectably label polyspecific immunoconjugates include β-galactosidase, glucose oxidase, peroxidase and alkaline phosphatase.

Those of skill in the art will know of other suitable labels, which can be employed in accordance with the present invention. The binding of marker moieties to anti-Zcytor21 antibodies can be accomplished using standard techniques known to the art. Typical methodology in this regard is described by Kennedy *et al., Clin. Chim. Acta 70*:1 (1976), Schurs *et al., Clin. Chim. Acta 81*:1 (1977), Shih *et al., Int'l J. Cancer 46*:1101 (1990), Stein *et al., Cancer Res. 50:*1330 (1990), and Coligan, *supra.*

Moreover, the convenience and versatility of immunochemical detection can be enhanced by using anti-Zcytor21 antibodies that have been conjugated with avidin, streptavidin, and biotin (see, for example, Wilchek *et al.* (eds.), "Avidin-Biotin Technology," *Methods In Enzymology, Vol. 184* (Academic Press 1990), and Bayer *et al.*, "Immunochemical Applications of Avidin-Biotin Technology," in *Methods In Molecular Biology, Vol. 10*, Manson (ed.), pages 149-162 (The Humana Press, Inc. 1992).

Methods for performing immunoassays are well-established. See, for example, Cook and Self, "Monoclonal Antibodies in Diagnostic Immunoassays," in *Monoclonal Antibodies: Production, Engineering, and Clinical Application*, Ritter and Ladyman (eds.), pages 180-208, (Cambridge University Press, 1995), Perry, "The Role of Monoclonal Antibodies in the Advancement of Immunoassay Technology," in *Monoclonal Antibodies: Principles and Applications*, Birch and Lennox (eds.), pages 107-120 (Wiley-Liss, Inc. 1995), and Diamandis, *Immunoassay* (Academic Press, Inc. 1996).

The present invention also contemplates kits for performing an immunological diagnostic assay for *Zcytor21* gene expression.. Such kits comprise at least one container comprising an anti-Zcytor21 antibody, or antibody fragment. A kit may also comprise a second container comprising one or more reagents capable of indicating the presence of Zcytor21 antibody or antibody fragments. Examples of such indicator reagents include detectable labels such as a radioactive label, a fluorescent

label, a chemiluminescent label, an enzyme label, a bioluminescent label, colloidal gold, and the like. A kit may also comprise a means for conveying to the user that Zcytor21 antibodies or antibody fragments are used to detect Zcytor21 protein. For example, written instructions may state that the enclosed antibody or antibody fragment can be used to detect Zcytor21. The written material can be applied directly to a container, or the written material can be provided in the form of a packaging insert.

11. Therapeutic Uses of Polypeptides Having Zcytor21 Activity

Amino acid sequences having Zcytor21 activity can be used to modulate the immune system by binding Zcytor21 ligand, and thus, preventing the binding of Zcytor21 ligand with endogenous Zcytor21 receptor. As an illustration, polypeptides having Zcytor21 activity can be used to inhibit cell proliferation associated with, for example, psoriasis or the growth of a tumor (*e.g.*, a melanoma). Zcytor21 antagonists, such as anti-Zcytor21 antibodies, can also be used to modulate the immune system by inhibiting the binding of Zcytor21 ligand with the endogenous Zcytor21 receptor.

Accordingly, the present invention includes the use of proteins, polypeptides, and peptides having Zcytor21 activity (such as Zcytor21 polypeptides, Zcytor21 analogs (*e.g.*, anti-Zcytor21 anti-idiotype antibodies), and Zcytor21 fusion proteins) to a subject which lacks an adequate amount of Zcytor21 polypeptide, or which produces an excess of Zcytor21 ligand. Zcytor21 antagonists (*e.g.*, anti-Zcytor21 antibodies) can be also used to treat a subject, which produces an excess of either Zcytor21 ligand or Zcytor21. These molecules can be administered to any subject in need of treatment, and the present invention contemplates both veterinary and human therapeutic uses. Illustrative subjects include mammalian subjects, such as farm animals, domestic animals, and human patients.

Generally, the dosage of administered Zcytor21 (or Zcytor21 analog or fusion protein) will vary depending upon such factors as the subject's age, weight, height, sex, general medical condition and previous medical history. Typically, it is desirable to provide the recipient with a dosage of Zcytor21 polypeptide, which is in the range of from about 1 pg/kg to 10 mg/kg (amount of agent/body weight of subject), although a lower or higher dosage also may be administered as circumstances dictate.

Administration of a Zcytor21 polypeptide to a subject can be intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, by perfusion through a regional catheter, or by direct intralesional injection. When administering therapeutic proteins by injection, the administration may be by continuous infusion or by single or multiple boluses.

Additional routes of administration include oral, mucosal-membrane, pulmonary, and transcutaneous. Oral delivery is suitable for polyester microspheres, zein microspheres, proteinoid microspheres, polycyanoacrylate microspheres, and lipid-based systems (see, for example, DiBase and Morrel, "Oral Delivery of Microencapsulated Proteins," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 255-288 (Plenum Press 1997)). The feasibility of an intranasal delivery is exemplified by such a mode of insulin administration (see, for example, Hinchcliffe and Illum, *Adv. Drug Deliv. Rev. 35*:199 (1999)). Dry or liquid particles comprising Zcytor21 can be prepared and inhaled with the aid of dry-powder dispersers, liquid aerosol generators, or nebulizers (*e.g.*, Pettit and Gombotz, *TIBTECH 16*:343 (1998); Patton *et al., Adv. Drug Deliv. Rev. 35*:235 (1999)). This approach is illustrated by the AERX diabetes management system, which is a hand-held electronic inhaler that delivers aerosolized insulin into the lungs. Studies have shown that proteins as large as 48,000 kDa have been delivered across skin at therapeutic concentrations with the aid of low-frequency ultrasound, which illustrates the feasibility of trascutaneous administration (Mitragotri *et al., Science 269*:850 (1995)). Transdermal delivery using electroporation provides another means to administer a molecule having Zcytor21 binding activity (Potts *et al., Pharm. Biotechnol. 10*:213 (1997)).

A pharmaceutical composition comprising a protein, polypeptide, or peptide having Zcytor21 binding activity can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic proteins are combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to

those in the art. See, for example, Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995).

For purposes of therapy, molecules having Zcytor21 binding activity and a pharmaceutically acceptable carrier are administered to a patient in a therapeutically effective amount. A combination of a protein, polypeptide, or peptide having Zcytor21 binding activity and a pharmaceutically acceptable carrier is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient. For example, an agent used to treat inflammation is physiologically significant if its presence alleviates the inflammatory response. As another example, an agent used to inhibit the growth of tumor cells is physiologically significant if the administration of the agent results in a decrease in the number of tumor cells, decreased metastasis, a decrease in the size of a solid tumor, or increased necrosis of a tumor.

A pharmaceutical composition comprising Zcytor21 (or Zcytor21 analog or fusion protein) can be furnished in liquid form, in an aerosol, or in solid form. Liquid forms, are illustrated by injectable solutions and oral suspensions. Exemplary solid forms include capsules, tablets, and controlled-release forms. The latter form is illustrated by miniosmotic pumps and implants (Bremer *et al., Pharm. Biotechnol. 10*:239 (1997); Ranade, "Implants in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 95-123 (CRC Press 1995); Bremer *et al.*, "Protein Delivery with Infusion Pumps," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 239-254 (Plenum Press 1997); Yewey *et al.*, "Delivery of Proteins from a Controlled Release Injectable Implant," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 93-117 (Plenum Press 1997)).

Liposomes provide one means to deliver therapeutic polypeptides to a subject intravenously, intraperitoneally, intrathecally, intramuscularly, subcutaneously, or via oral administration, inhalation, or intranasal administration. Liposomes are microscopic vesicles that consist of one or more lipid bilayers surrounding aqueous compartments (see, generally, Bakker-Woudenberg *et al., Eur. J. Clin. Microbiol. Infect. Dis. 12 (Suppl. 1)*:S61 (1993), Kim, *Drugs 46*:618 (1993), and Ranade, "Site-

Specific Drug Delivery Using Liposomes as Carriers," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 3-24 (CRC Press 1995)). Liposomes are similar in composition to cellular membranes and as a result, liposomes can be administered safely and are biodegradable. Depending on the method of preparation, liposomes may be unilamellar or multilamellar, and liposomes can vary in size with diameters ranging from 0.02 μm to greater than 10 μm. A variety of agents can be encapsulated in liposomes: hydrophobic agents partition in the bilayers and hydrophilic agents partition within the inner aqueous space(s) (see, for example, Machy *et al., Liposomes In Cell Biology And Pharmacology* (John Libbey 1987), and Ostro *et al., American J. Hosp. Pharm. 46*:1576 (1989)). Moreover, it is possible to control the therapeutic availability of the encapsulated agent by varying liposome size, the number of bilayers, lipid composition, as well as the charge and surface characteristics of the liposomes.

Liposomes can adsorb to virtually any type of cell and then slowly release the encapsulated agent. Alternatively, an absorbed liposome may be endocytosed by cells that are phagocytic. Endocytosis is followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents (Scherphof *et al., Ann. N.Y. Acad. Sci. 446*:368 (1985)). After intravenous administration, small liposomes (0.1 to 1.0 μm) are typically taken up by cells of the reticuloendothelial system, located principally in the liver and spleen, whereas liposomes larger than 3.0 μm are deposited in the lung. This preferential uptake of smaller liposomes by the cells of the reticuloendothelial system has been used to deliver chemotherapeutic agents to macrophages and to tumors of the liver.

The reticuloendothelial system can be circumvented by several methods including saturation with large doses of liposome particles, or selective macrophage inactivation by pharmacological means (Claassen *et al., Biochim. Biophys. Acta 802*:428 (1984)). In addition, incorporation of glycolipid- or polyethelene glycol-derivatized phospholipids into liposome membranes has been shown to result in a significantly reduced uptake by the reticuloendothelial system (Allen *et al., Biochim. Biophys. Acta 1068*:133 (1991); Allen *et al., Biochim. Biophys. Acta 1150*:9 (1993)).

Liposomes can also be prepared to target particular cells or organs by varying phospholipid composition or by inserting receptors or ligands into the

liposomes. For example, liposomes, prepared with a high content of a nonionic surfactant, have been used to target the liver (Hayakawa *et al.*, Japanese Patent 04-244,018; Kato *et al., Biol. Pharm. Bull. 16*:960 (1993)). These formulations were prepared by mixing soybean phospatidylcholine, α-tocopherol, and ethoxylated hydrogenated castor oil (HCO-60) in methanol, concentrating the mixture under vacuum, and then reconstituting the mixture with water. A liposomal formulation of dipalmitoylphosphatidylcholine (DPPC) with a soybean-derived sterylglucoside mixture (SG) and cholesterol (Ch) has also been shown to target the liver (Shimizu *et al., Biol. Pharm. Bull. 20*:881 (1997)).

Alternatively, various targeting ligands can be bound to the surface of the liposome, such as antibodies, antibody fragments, carbohydrates, vitamins, and transport proteins. For example, liposomes can be modified with branched type galactosyllipid derivatives to target asialoglycoprotein (galactose) receptors, which are exclusively expressed on the surface of liver cells (Kato and Sugiyama, *Crit. Rev. Ther. Drug Carrier Syst. 14*:287 (1997); Murahashi *et al., Biol. Pharm. Bull.20*:259 (1997)). Similarly, Wu *et al., Hepatology 27*:772 (1998), have shown that labeling liposomes with asialofetuin led to a shortened liposome plasma half-life and greatly enhanced uptake of asialofetuin-labeled liposome by hepatocytes. On the other hand, hepatic accumulation of liposomes comprising branched type galactosyllipid derivatives can be inhibited by preinjection of asialofetuin (Murahashi *et al., Biol. Pharm. Bull.20*:259 (1997)). Polyaconitylated human serum albumin liposomes provide another approach for targeting liposomes to liver cells (Kamps *et al., Proc. Nat'l Acad. Sci. USA 94*:11681 (1997)). Moreover, Geho, *et al.* U.S. Patent No. 4,603,044, describe a hepatocyte-directed liposome vesicle delivery system, which has specificity for hepatobiliary receptors associated with the specialized metabolic cells of the liver.

In a more general approach to tissue targeting, target cells are prelabeled with biotinylated antibodies specific for a ligand expressed by the target cell (Harasym *et al., Adv. Drug Deliv. Rev. 32*:99 (1998)). After plasma elimination of free antibody, streptavidin-conjugated liposomes are administered. In another approach, targeting antibodies are directly attached to liposomes (Harasym *et al., Adv. Drug Deliv. Rev. 32*:99 (1998)).

Polypeptides having Zcytor21 binding activity can be encapsulated within liposomes using standard techniques of protein microencapsulation (see, for example, Anderson *et al., Infect. Immun. 31*:1099 (1981), Anderson *et al., Cancer Res. 50*:1853 (1990), and Cohen *et al., Biochim. Biophys. Acta 1063*:95 (1991), Alving *et al.* "Preparation and Use of Liposomes in Immunological Studies," in *Liposome Technology,* 2nd Edition, Vol. III, Gregoriadis (ed.), page 317 (CRC Press 1993), Wassef *et al., Meth. Enzymol. 149*:124 (1987)). As noted above, therapeutically useful liposomes may contain a variety of components. For example, liposomes may comprise lipid derivatives of poly(ethylene glycol) (Allen *et al., Biochim. Biophys. Acta 1150*:9 (1993)).

Degradable polymer microspheres have been designed to maintain high systemic levels of therapeutic proteins. Microspheres are prepared from degradable polymers such as poly(lactide-co-glycolide) (PLG), polyanhydrides, poly (ortho esters), nonbiodegradable ethylvinyl acetate polymers, in which proteins are entrapped in the polymer (Gombotz and Pettit, *Bioconjugate Chem.* 6:332 (1995); Ranade, "Role of Polymers in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 51-93 (CRC Press 1995); Roskos and Maskiewicz, "Degradable Controlled Release Systems Useful for Protein Delivery," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 45-92 (Plenum Press 1997); Bartus *et al., Science 281*:1161 (1998); Putney and Burke, *Nature Biotechnology 16*:153 (1998); Putney, *Curr. Opin. Chem. Biol.* 2:548 (1998)). Polyethylene glycol (PEG)-coated nanospheres can also provide carriers for intravenous administration of therapeutic proteins (see, for example, Gref *et al., Pharm. Biotechnol. 10*:167 (1997)).

The present invention also contemplates chemically modified polypeptides having binding Zcytor21 activity and Zcytor21 antagonists, in which a polypeptide is linked with a polymer, as discussed above.

Other dosage forms can be devised by those skilled in the art, as shown, for example, by Ansel and Popovich, *Pharmaceutical Dosage Forms and Drug Delivery Systems,* 5th Edition (Lea & Febiger 1990), Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995), and by Ranade and Hollinger, *Drug Delivery Systems* (CRC Press 1996).

As an illustration, pharmaceutical compositions may be supplied as a kit comprising a container that comprises a polypeptide with a Zcytor21 extracellular domain or a Zcytor21 antagonist (*e.g.*, an antibody or antibody fragment that binds a Zcytor21 polypeptide). Therapeutic polypeptides can be provided in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a therapeutic polypeptide. Such a kit may further comprise written information on indications and usage of the pharmaceutical composition. Moreover, such information may include a statement that the Zcytor21 composition is contraindicated in patients with known hypersensitivity to Zcytor21.

12. Therapeutic Uses of Zcytor21 Nucleotide Sequences

The present invention includes the use of *Zcytor21* nucleotide sequences to provide Zcytor21 to a subject in need of such treatment. In addition, a therapeutic expression vector can be provided that inhibits *Zcytor21* gene expression, such as an anti-sense molecule, a ribozyme, or an external guide sequence molecule.

There are numerous approaches to introduce a *Zcytor21* gene to a subject, including the use of recombinant host cells that express *Zcytor21*, delivery of naked nucleic acid encoding Zcytor21, use of a cationic lipid carrier with a nucleic acid molecule that encodes Zcytor21, and the use of viruses that express *Zcytor21*, such as recombinant retroviruses, recombinant adeno-associated viruses, recombinant adenoviruses, and recombinant Herpes simplex viruses (see, for example, Mulligan, *Science 260*:926 (1993), Rosenberg *et al., Science 242*:1575 (1988), LaSalle *et al., Science 259*:988 (1993), Wolff *et al., Science 247*:1465 (1990), Breakfield and Deluca, *The New Biologist 3*:203 (1991)). In an *ex vivo* approach, for example, cells are isolated from a subject, transfected with a vector that expresses a *Zcytor21* gene, and then transplanted into the subject.

In order to effect expression of a *Zcytor21* gene, an expression vector is constructed in which a nucleotide sequence encoding a *Zcytor21* gene is operably linked

to a core promoter, and optionally a regulatory element, to control gene transcription. The general requirements of an expression vector are described above.

Alternatively, a *Zcytor21* gene can be delivered using recombinant viral vectors, including for example, adenoviral vectors (*e.g.*, Kass-Eisler *et al., Proc. Nat'l Acad. Sci. USA 90*:11498 (1993), Kolls *et al., Proc. Nat'l Acad. Sci. USA 91*:215 (1994), Li *et al., Hum. Gene Ther. 4*:403 (1993), Vincent *et al., Nat. Genet. 5*:130 (1993), and Zabner *et al., Cell 75*:207 (1993)), adenovirus-associated viral vectors (Flotte *et al., Proc. Nat'l Acad. Sci. USA 90*:10613 (1993)), alphaviruses such as Semliki Forest Virus and Sindbis Virus (Hertz and Huang, *J. Vir. 66*:857 (1992), Raju and Huang, *J. Vir. 65*:2501 (1991), and Xiong *et al., Science* 243:1188 (1989)), herpes viral vectors (*e.g.*, U.S. Patent Nos. 4,769,331, 4,859,587, 5,288,641 and 5,328,688), parvovirus vectors (Koering *et al., Hum. Gene Therap. 5*:457 (1994)), pox virus vectors (Ozaki *et al., Biochem. Biophys. Res. Comm. 193*:653 (1993), Panicali and Paoletti, *Proc. Nat'l Acad. Sci. USA 79*:4927 (1982)), pox viruses, such as canary pox virus or vaccinia virus (Fisher-Hoch *et al., Proc. Nat'l Acad. Sci. USA 86*:317 (1989), and Flexner *et al., Ann. N.Y. Acad. Sci. 569*:86 (1989)), and retroviruses (*e.g.*, Baba *et al., J. Neurosurg 79*:729 (1993), Ram *et al., Cancer Res. 53*:83 (1993), Takamiya *et al., J. Neurosci. Res 33*:493 (1992), Vile and Hart, *Cancer Res. 53*:962 (1993), Vile and Hart, *Cancer Res. 53*:3860 (1993), and Anderson *et al.*, U.S. Patent No. 5,399,346). Within various embodiments, either the viral vector itself, or a viral particle which contains the viral vector may be utilized in the methods and compositions described below.

As an illustration of one system, adenovirus, a double-stranded DNA virus, is a well-characterized gene transfer vector for delivery of a heterologous nucleic acid molecule (for a review, see Becker *et al., Meth. Cell Biol. 43*:161 (1994); Douglas and Curiel, *Science & Medicine 4*:44 (1997)). The adenovirus system offers several advantages including: (i) the ability to accommodate relatively large DNA inserts, (ii) the ability to be grown to high-titer, (iii) the ability to infect a broad range of mammalian cell types, and (iv) the ability to be used with many different promoters including ubiquitous, tissue specific, and regulatable promoters. In addition, adenoviruses can be administered by intravenous injection, because the viruses are stable in the bloodstream.

Using adenovirus vectors where portions of the adenovirus genome are deleted, inserts are incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. In an exemplary system, the essential E1 gene is deleted from the viral vector, and the virus will not replicate unless the E1 gene is provided by the host cell. When intravenously administered to intact animals, adenovirus primarily targets the liver. Although an adenoviral delivery system with an E1 gene deletion cannot replicate in the host cells, the host's tissue will express and process an encoded heterologous protein. Host cells will also secrete the heterologous protein if the corresponding gene includes a secretory signal sequence. Secreted proteins will enter the circulation from tissue that expresses the heterologous gene (*e.g.*, the highly vascularized liver).

Moreover, adenoviral vectors containing various deletions of viral genes can be used to reduce or eliminate immune responses to the vector. Such adenoviruses are E1-deleted, and in addition, contain deletions of E2A or E4 (Lusky *et al., J. Virol. 72*:2022 (1998); Raper *et al., Human Gene Therapy 9*:671 (1998)). The deletion of E2b has also been reported to reduce immune responses (Amalfitano *et al., J. Virol. 72*:926 (1998)). By deleting the entire adenovirus genome, very large inserts of heterologous DNA can be accommodated. The generation of so called "gutless" adenoviruses, where all viral genes are deleted, is particularly advantageous for insertion of large inserts of heterologous DNA (for a review, see Yeh. and Perricaudet, *FASEB J. 11*:615 (1997)).

High titer stocks of recombinant viruses capable of expressing a therapeutic gene can be obtained from infected mammalian cells using standard methods. For example, recombinant herpes simplex virus can be prepared in Vero cells, as described by Brandt *et al., J. Gen. Virol. 72*:2043 (1991), Herold *et al., J. Gen. Virol. 75*:1211 (1994), Visalli and Brandt, *Virology 185*:419 (1991), Grau *et al., Invest. Ophthalmol. Vis. Sci. 30*:2474 (1989), Brandt *et al., J. Virol. Meth. 36*:209 (1992), and by Brown and MacLean (eds.), *HSV Virus Protocols* (Humana Press 1997).

Alternatively, an expression vector comprising a *Zcytor21* gene can be introduced into a subject's cells by lipofection *in vivo* using liposomes. Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner *et al., Proc. Nat'l Acad. Sci. USA 84*:7413 (1987); Mackey

et al., Proc. Nat'l Acad. Sci. USA 85:8027 (1988)). The use of lipofection to introduce exogenous genes into specific organs *in vivo* has certain practical advantages. Liposomes can be used to direct transfection to particular cell types, which is particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides (*e.g.*, hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

Electroporation is another alternative mode of administration. For example, Aihara and Miyazaki, *Nature Biotechnology 16*:867 (1998), have demonstrated the use of *in vivo* electroporation for gene transfer into muscle.

In an alternative approach to gene therapy, a therapeutic gene may encode a *Zcytor21* anti-sense RNA that inhibits the expression of *Zcytor21*. Suitable sequences for anti-sense molecules can be derived from the nucleotide sequences of *Zcytor21* disclosed herein.

Alternatively, an expression vector can be constructed in which a regulatory element is operably linked to a nucleotide sequence that encodes a ribozyme. Ribozymes can be designed to express endonuclease activity that is directed to a certain target sequence in an mRNA molecule (see, for example, Draper and Macejak, U.S. Patent No. 5,496,698, McSwiggen, U.S. Patent No. 5,525,468, Chowrira and McSwiggen, U.S. Patent No. 5,631,359, and Robertson and Goldberg, U.S. Patent No. 5,225,337). In the context of the present invention, ribozymes include nucleotide sequences that bind with *Zcytor21* mRNA.

In another approach, expression vectors can be constructed in which a regulatory element directs the production of RNA transcripts capable of promoting RNase P-mediated cleavage of mRNA molecules that encode a *Zcytor21* gene. According to this approach, an external guide sequence can be constructed for directing the endogenous ribozyme, RNase P, to a particular species of intracellular mRNA, which is subsequently cleaved by the cellular ribozyme (see, for example, Altman *et al.*, U.S. Patent No. 5,168,053, Yuan *et al.*, *Science 263*:1269 (1994), Pace *et al.*, international publication No. WO 96/18733, George *et al.*, international publication No. WO 96/21731, and Werner *et al.*, international publication No. WO 97/33991). For example, the external

guide sequence can comprise a ten to fifteen nucleotide sequence complementary to *Zcytor21* mRNA, and a 3'-NCCA nucleotide sequence, wherein N is preferably a purine. The external guide sequence transcripts bind to the targeted mRNA species by the formation of base pairs between the mRNA and the complementary external guide sequences, thus promoting cleavage of mRNA by RNase P at the nucleotide located at the 5'-side of the base-paired region.

In general, the dosage of a composition comprising a therapeutic vector having a *Zcytor21* nucleotide sequence, such as a recombinant virus, will vary depending upon such factors as the subject's age, weight, height, sex, general medical condition and previous medical history. Suitable routes of administration of therapeutic vectors include intravenous injection, intraarterial injection, intraperitoneal injection, intramuscular injection, intratumoral injection, and injection into a cavity that contains a tumor. As an illustration, Horton *et al., Proc. Nat'l Acad. Sci. USA 96*:1553 (1999), demonstrated that intramuscular injection of plasmid DNA encoding interferon-α produces potent antitumor effects on primary and metastatic tumors in a murine model.

A composition comprising viral vectors, non-viral vectors, or a combination of viral and non-viral vectors of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby vectors or viruses are combined in a mixture with a pharmaceutically acceptable carrier. As noted above, a composition, such as phosphate-buffered saline is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient subject. Other suitable carriers are well-known to those in the art (see, for example, *Remington's Pharmaceutical Sciences, 19th Ed.* (Mack Publishing Co. 1995), and *Gilman's the Pharmacological Basis of Therapeutics, 7th Ed.* (MacMillan Publishing Co. 1985)).

For purposes of therapy, a therapeutic gene expression vector, or a recombinant virus comprising such a vector, and a pharmaceutically acceptable carrier are administered to a subject in a therapeutically effective amount. A combination of an expression vector (or virus) and a pharmaceutically acceptable carrier is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence

results in a detectable change in the physiology of a recipient subject. For example, an agent used to treat inflammation is physiologically significant if its presence alleviates the inflammatory response.

When the subject treated with a therapeutic gene expression vector or a recombinant virus is a human, then the therapy is preferably somatic cell gene therapy. That is, the preferred treatment of a human with a therapeutic gene expression vector or a recombinant virus does not entail introducing into cells a nucleic acid molecule that can form part of a human germ line and be passed onto successive generations (*i.e.*, human germ line gene therapy).

13. Production of Transgenic Mice

Transgenic mice can be engineered to over-express the *Zcytor21* gene in all tissues or under the control of a tissue-specific or tissue-preferred regulatory element. These over-producers of Zcytor21 can be used to characterize the phenotype that results from over-expression, and the transgenic animals can serve as models for human disease caused by excess Zcytor21. Transgenic mice that over-express Zcytor21 also provide model bioreactors for production of Zcytor21, such as soluble Zcytor21, in the milk or blood of larger animals. Methods for producing transgenic mice are well-known to those of skill in the art (see, for example, Jacob, "Expression and Knockout of Interferons in Transgenic Mice," in *Overexpression and Knockout of Cytokines in Transgenic Mice*, Jacob (ed.), pages 111-124 (Academic Press, Ltd. 1994), Monastersky and Robl (eds.), *Strategies in Transgenic Animal Science* (ASM Press 1995), and Abbud and Nilson, "Recombinant Protein Expression in Transgenic Mice," in *Gene Expression Systems: Using Nature for the Art of Expression*, Fernandez and Hoeffler (eds.), pages 367-397 (Academic Press, Inc. 1999)).

For example, a method for producing a transgenic mouse that expresses a *Zcytor21* gene can begin with adult, fertile males (studs) (B6C3f1, 2-8 months of age (Taconic Farms, Germantown, NY)), vasectomized males (duds) (B6D2f1, 2-8 months, (Taconic Farms)), prepubescent fertile females (donors) (B6C3f1, 4-5 weeks, (Taconic Farms)) and adult fertile females (recipients) (B6D2f1, 2-4 months, (Taconic Farms)). The donors are acclimated for one week and then injected with approximately 8

IU/mouse of Pregnant Mare's Serum gonadotrophin (Sigma Chemical Company; St. Louis, MO) I.P., and 46-47 hours later, 8 IU/mouse of human Chorionic Gonadotropin (hCG (Sigma)) I.P. to induce superovulation. Donors are mated with studs subsequent to hormone injections. Ovulation generally occurs within 13 hours of hCG injection. Copulation is confirmed by the presence of a vaginal plug the morning following mating.

Fertilized eggs are collected under a surgical scope. The oviducts are collected and eggs are released into urinanalysis slides containing hyaluronidase (Sigma). Eggs are washed once in hyaluronidase, and twice in Whitten's W640 medium (described, for example, by Menino and O'Claray, *Biol. Reprod.* *77*:159 (1986), and Dienhart and Downs, *Zygote* *4*:129 (1996)) that has been incubated with 5% CO_2, 5% O_2, and 90% N_2 at 37°C. The eggs are then stored in a 37°C/5% CO_2 incubator until microinjection.

Ten to twenty micrograms of plasmid DNA containing a *Zcytor21* encoding sequence is linearized, gel-purified, and resuspended in 10 mM Tris-HCl (pH 7.4), 0.25 mM EDTA (pH 8.0), at a final concentration of 5-10 nanograms per microliter for microinjection. For example, the *Zcytor21* encoding sequences can encode a polypeptide comprising amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444 of SEQ ID NO:15.

Plasmid DNA is microinjected into harvested eggs contained in a drop of W640 medium overlaid by warm, CO_2-equilibrated mineral oil. The DNA is drawn into an injection needle (pulled from a 0.75mm ID, 1mm OD borosilicate glass capillary), and injected into individual eggs. Each egg is penetrated with the injection needle, into one or both of the haploid pronuclei.

Picoliters of DNA are injected into the pronuclei, and the injection needle withdrawn without coming into contact with the nucleoli. The procedure is repeated until all the eggs are injected. Successfully microinjected eggs are transferred into an organ tissue-culture dish with pre-gassed W640 medium for storage overnight in a 37°C/5% CO_2 incubator.

The following day, two-cell embryos are transferred into pseudopregnant recipients. The recipients are identified by the presence of copulation plugs, after copulating with vasectomized duds. Recipients are anesthetized and shaved on the dorsal left side and transferred to a surgical microscope. A small incision is made in the skin and through the muscle wall in the middle of the abdominal area outlined by the ribcage, the saddle, and the hind leg, midway between knee and spleen. The reproductive organs are exteriorized onto a small surgical drape. The fat pad is stretched out over the surgical drape, and a baby serrefine (Roboz, Rockville, MD) is attached to the fat pad and left hanging over the back of the mouse, preventing the organs from sliding back in.

With a fine transfer pipette containing mineral oil followed by alternating W640 and air bubbles, 12-17 healthy two-cell embryos from the previous day's injection are transferred into the recipient. The swollen ampulla is located and holding the oviduct between the ampulla and the bursa, a nick in the oviduct is made with a 28 g needle close to the bursa, making sure not to tear the ampulla or the bursa.

The pipette is transferred into the nick in the oviduct, and the embryos are blown in, allowing the first air bubble to escape the pipette. The fat pad is gently pushed into the peritoneum, and the reproductive organs allowed to slide in. The peritoneal wall is closed with one suture and the skin closed with a wound clip. The mice recuperate on a 37°C slide warmer for a minimum of four hours.

The recipients are returned to cages in pairs, and allowed 19-21 days gestation. After birth, 19-21 days postpartum is allowed before weaning. The weanlings are sexed and placed into separate sex cages, and a 0.5 cm biopsy (used for genotyping) is snipped off the tail with clean scissors.

Genomic DNA is prepared from the tail snips using, for example, a QIAGEN DNEASY kit following the manufacturer's instructions. Genomic DNA is analyzed by PCR using primers designed to amplify a *Zcytor21* gene or a selectable marker gene that was introduced in the same plasmid. After animals are confirmed to be transgenic, they are back-crossed into an inbred strain by placing a transgenic female with a wild-type male, or a transgenic male with one or two wild-type female(s). As

pups are born and weaned, the sexes are separated, and their tails snipped for genotyping.

To check for expression of a transgene in a live animal, a partial hepatectomy is performed. A surgical prep is made of the upper abdomen directly below the zyphoid process. Using sterile technique, a small 1.5-2 cm incision is made below the sternum and the left lateral lobe of the liver exteriorized. Using 4-0 silk, a tie is made around the lower lobe securing it outside the body cavity. An atraumatic clamp is used to hold the tie while a second loop of absorbable Dexon (American Cyanamid; Wayne, N.J.) is placed proximal to the first tie. A distal cut is made from the Dexon tie and approximately 100 mg of the excised liver tissue is placed in a sterile petri dish. The excised liver section is transferred to a 14 ml polypropylene round bottom tube and snap frozen in liquid nitrogen and then stored on dry ice. The surgical site is closed with suture and wound clips, and the animal's cage placed on a 37°C heating pad for 24 hours post operatively. The animal is checked daily post operatively and the wound clips removed 7-10 days after surgery. The expression level of Zcytor21 mRNA is examined for each transgenic mouse using an RNA solution hybridization assay or polymerase chain reaction.

In addition to producing transgenic mice that over-express Zcytor21, it is useful to engineer transgenic mice with either abnormally low or no expression of the gene. Such transgenic mice provide useful models for diseases associated with a lack of Zcytor21. As discussed above, *Zcytor21* gene expression can be inhibited using anti-sense genes, ribozyme genes, or external guide sequence genes. To produce transgenic mice that under-express the *Zcytor21* gene, such inhibitory sequences are targeted to *Zcytor21* mRNA. Methods for producing transgenic mice that have abnormally low expression of a particular gene are known to those in the art (see, for example, Wu *et al.*, "Gene Underexpression in Cultured Cells and Animals by Antisense DNA and RNA Strategies," in *Methods in Gene Biotechnology*, pages 205-224 (CRC Press 1997)).

An alternative approach to producing transgenic mice that have little or no *Zcytor21* gene expression is to generate mice having at least one normal *Zcytor21* allele replaced by a nonfunctional *Zcytor21* gene. One method of designing a

nonfunctional *Zcytor21* gene is to insert another gene, such as a selectable marker gene, within a nucleic acid molecule that encodes Zcytor21. Standard methods for producing these so-called "knockout mice" are known to those skilled in the art (see, for example, Jacob, "Expression and Knockout of Interferons in Transgenic Mice," in *Overexpression and Knockout of Cytokines in Transgenic Mice*, Jacob (ed.), pages 111-124 (Academic Press, Ltd. 1994), and Wu *et al.*, "New Strategies for Gene Knockout," in *Methods in Gene Biotechnology*, pages 339-365 (CRC Press 1997)).

Example 1

Human Zcytor21 Tissue Distribution in Tissue Panels Using PCR

Tissue Distribution in tissue cDNA panels using PCR

The Human Rapid-Scan cDNA panel represents 24 adult tissues and is arrayed at 4 different concentrations called 1X, 10X, 100X, and 1000X (Origen, Rockville, MD.). The "1000x and 100x" levels were screened for zcytor21 transcription using PCR. The sense primer was zc39334, (5' AGGCCCTGCCACCCACCTTC 3') (SEQ ID NO:17) located in a cDNA area corresponding to the 5' untranslated region. The antisense primer was zc39333, (5'-CGAGGCACCCCAAGGATTTCAG-3') (SEQ ID NO:18) located in a cDNA area corresponding to the 3' untranslated region. PCR was applied using pfu turbo polymerase and the manufacturer's recommendations (Stratagene, La Jolla, CA) except for using rediload dye, (Research Genetics, Inc., Huntsville, AL) a wax hot start, (Molecular Bioproducts Inc. San Diego, CA) and 10% (final concentration) DMSO. The amplification was carried out as follows: 1 cycle at 94°C for 4 minutes, 40 cycles of 94°C for 30 seconds, 51°C for 30 seconds and 72°C for 3 minutes, followed by 1 cycle at 72°C for 7 minutes. About 10 µl of the PCR reaction product was subjected to standard agarose gel electrophoresis using a 1% agarose gel. Following electrophoresis, the gels were Southern blotted and the membranes hybridized by standard methods using a ^{32}P isotope-labeled oligonucleotide, zc40458 (5'-TCTCTGACTCTGCTGGGATTGG-3') (SEQ ID NO:19) which maps to the cDNA area in the translated region, just downstream of the start codon. X ray film

autoradiography revealed zcytor21-specific amplicons only in colon, lung, stomach, placenta, and bone marrow.

The complete disclosure of all patents, patent applications, and publications, and electronically available material (e.g., GenBank amino acid and nucleotide sequence submissions) cited herein are incorporated by reference. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

CLAIMS

What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, and amino acid residues 24 to 444 of SEQ ID NO:15.

2. The isolated polypeptide of claim 1 wherein the isolated polypeptide comprises an amino acid sequence selected from the group consisting of amino acid residues 24 to 667 of SEQ ID NO:11, amino acid residues 24 to 589 of SEQ ID NO:2, amino acid residues 24 to 609 of SEQ ID NO:5, and amino acid residues 24 to 657 of SEQ ID NO:15.

3. The isolated polypeptide of claim 1 wherein the isolated polypeptide comprises an amino acid sequence selected from the group consisting of the amino acid sequence of SEQ ID NO:2, the amino acid sequence of SEQ ID NO:5, the amino acid sequence of SEQ ID NO:8, the amino acid sequence of SEQ ID NO:11, and the amino acid sequence of SEQ ID NO:15.

4. An isolated nucleic acid molecule encoding a polypeptide wherein the polypeptide comprises an amino acid sequence selected from the group consisting of a amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, and amino acid residues 24 to 444 of SEQ ID NO:15.

5. A vector comprising the isolated nucleic acid molecule of claim 4.

6. An expression vector comprising the isolated nucleic acid molecule of claim 4, a transcription promoter, and a transcription terminator, wherein the promoter is operably

linked with the nucleic acid molecule, and wherein the nucleic acid molecule is operably linked with the transcription terminator.

7. A recombinant host cell comprising the expression vector of claim 6 wherein the host cell is selected from the group consisting of bacterium, yeast cell, fungal cell, insect cell, avian cell, mammalian cell, and plant cell.

8. A method of producing Zcytor21 protein, the method comprising:

culturing recombinant host cells that comprise the expression vector of claim 6, and that produce the Zcytor21 protein.

9. The method of claim 8 further comprising isolating the Zcytor21 protein from the cultured recombinant host cells.

10. An antibody or antibody fragment that specifically binds with the polypeptide of claim 1.

11. The antibody of claim 10 wherein the antibody is selected from the group consisting of a polyclonal antibody, a murine monoclonal antibody, a humanized antibody derived from a murine monoclonal antibody, and a human monoclonal antibody.

12. An anti-idiotype antibody that specifically binds with the antibody of claim 10.

13. A fusion protein comprising the polypeptide of claim 1.

14. The fusion protein of claim 13 wherein the fusion protein further comprises an immunoglobulin moiety.

15. A composition comprising:

an effective amount of the polypeptide of claim 1; and

a pharmaceutically acceptable vehicle.

SEQUENCE LISTING

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<120> Human Cytokine Receptor

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Pro His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Arg Lys Leu
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Gln Lys Ile Val Ser Gly Gly His Thr Val Glu Leu Pro Tyr Glu Phe
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ctt ctg ccc tgt ctg tgc ata gag gca tcc tac ctg caa gag gac act      590
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160                 165                 170                 175

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305 310 315 320
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80                  85                  90                  95

cct ccc cag aca tct ccc aca agg gac ttc gct cta aaa gga ccc aac    398
Pro Pro Gln Thr Ser Pro Thr Arg Asp Phe Ala Leu Lys Gly Pro Asn
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Leu Arg Ile Gln Arg His Gly Lys Val Phe Pro Asp Trp Thr His Lys
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gga ccc gag ttc tcc ttt gat ttg ctg cct gag gcc cgg gct att cgg    494
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Trp Ala Leu Glu Cys Glu Glu Leu Ser Ser Pro Tyr Asp Val Gln Lys
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att gtg tct ggg ggc cac act gta gag ctg cct tat gaa ttc ctt ctg    638
Ile Val Ser Gly Gly His Thr Val Glu Leu Pro Tyr Glu Phe Leu Leu
                180                 185                 190

ccc tgt ctg tgc ata gag gca tcc tac ctg caa gag gac act gtg agg    686
Pro Cys Leu Cys Ile Glu Ala Ser Tyr Leu Gln Glu Asp Thr Val Arg
            195                 200                 205

cgc aaa aaa tgt ccc ttc cag agc tgg cca gaa gcc tat ggc tcg gac    734
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560 565 570 575

gcc acc agc tgg ggc cgc ctt ggg gcg cgg cag cgc agg cag agc cgc 1838
Ala Thr Ser Trp Gly Arg Leu Gly Ala Arg Gln Arg Arg Gln Ser Arg
580 585 590

cta gag ctg tgc agc cgg ctc gaa cga gag gcc gcc cga ctt gca gac 1886
Leu Glu Leu Cys Ser Arg Leu Glu Arg Glu Ala Ala Arg Leu Ala Asp
595 600 605

cta ggt tgagcagagc tccaccgcag tcccgggtgt ctgcggccgc aacgcaacgg 1942
Leu Gly

acactggctg gaaccccgga atgagccttc gaccctgaaa tccttggggt gcctcg 1998

<210> 5

<211> 609
<212> PRT
<213> Homo sapiens

<400> 5
Met Gly Ser Ser Arg Leu Ala Ala Leu Leu Leu Pro Leu Leu Leu Ile
1 5 10 15
Val Ile Asp Leu Ser Asp Ser Ala Gly Ile Gly Phe Arg His Leu Pro
20 25 30
His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Val Phe Asn Gly
35 40 45
Ala Ser Ser Thr Ser Trp Cys Arg Asn Pro Lys Ser Leu Pro His Ser
50 55 60
Ser Ser Ile Gly Asp Thr Arg Cys Gln His Leu Leu Arg Gly Ser Cys
65 70 75 80
Cys Leu Val Val Thr Cys Leu Arg Arg Ala Ile Thr Phe Pro Ser Pro
85 90 95
Pro Gln Thr Ser Pro Thr Arg Asp Phe Ala Leu Lys Gly Pro Asn Leu
100 105 110
Arg Ile Gln Arg His Gly Lys Val Phe Pro Asp Trp Thr His Lys Gly
115 120 125
Pro Glu Phe Ser Phe Asp Leu Leu Pro Glu Ala Arg Ala Ile Arg Val
130 135 140
Thr Ile Ser Ser Gly Pro Glu Val Ser Val Arg Leu Cys His Gln Trp
145 150 155 160
Ala Leu Glu Cys Glu Glu Leu Ser Ser Pro Tyr Asp Val Gln Lys Ile
165 170 175
Val Ser Gly Gly His Thr Val Glu Leu Pro Tyr Glu Phe Leu Leu Pro
180 185 190
Cys Leu Cys Ile Glu Ala Ser Tyr Leu Gln Glu Asp Thr Val Arg Arg
195 200 205
Lys Lys Cys Pro Phe Gln Ser Trp Pro Glu Ala Tyr Gly Ser Asp Phe
210 215 220
Trp Lys Ser Val His Phe Thr Asp Tyr Ser Gln His Thr Gln Met Val
225 230 235 240
Met Ala Leu Thr Leu Arg Cys Pro Leu Lys Leu Glu Ala Ala Leu Cys
245 250 255
Gln Arg His Asp Trp His Thr Leu Cys Lys Asp Leu Pro Asn Ala Thr
260 265 270
Ala Arg Glu Ser Asp Gly Trp Tyr Val Leu Glu Lys Val Asp Leu His
275 280 285
Pro Gln Leu Cys Phe Lys Phe Ser Phe Gly Asn Ser Ser His Val Glu
290 295 300
Cys Pro His Gln Thr Gly Ile Thr Glu Ala Arg Asp Trp Pro Ser His
305 310 315 320
Ile Gln Val Ser Cys Ser Pro Gly Val Pro Ile Arg Glu Pro Gln Thr
325 330 335
Ser Asn Cys Leu Trp Phe Val Arg Asn Glu Ala Thr Gln Gln Glu Ala
340 345 350
Arg Gly Ser Ser Pro Val Ser Leu Asp Leu Ile Ile Pro Phe Leu Arg
355 360 365
Pro Gly Cys Cys Val Leu Val Trp Arg Ser Asp Val Gln Phe Ala Trp
370 375 380
Lys His Leu Leu Cys Pro Asp Val Ser Tyr Arg His Leu Gly Leu Leu
385 390 395 400
Ile Leu Ala Leu Leu Ala Leu Leu Thr Leu Leu Gly Val Val Leu Ala
405 410 415
Leu Thr Cys Arg Arg Pro Gln Ser Gly Pro Gly Pro Ala Arg Pro Val
420 425 430
Leu Leu Leu His Ala Ala Asp Ser Glu Ala Gln Arg Arg Leu Val Gly
435 440 445

```
Ala Leu Ala Glu Leu Leu Arg Ala Ala Leu Gly Gly Gly Arg Asp Val
    450                 455                 460
Ile Val Asp Leu Trp Glu Gly Arg His Val Ala Arg Val Gly Pro Leu
465                 470                 475                 480
Pro Trp Leu Trp Ala Ala Arg Thr Arg Val Ala Arg Glu Gln Gly Thr
                485                 490                 495
Val Leu Leu Leu Trp Ser Gly Ala Asp Leu Arg Pro Val Ser Gly Pro
            500                 505                 510
Asp Pro Arg Ala Ala Pro Leu Leu Ala Leu Leu His Ala Ala Pro Arg
        515                 520                 525
Pro Leu Leu Leu Leu Ala Tyr Phe Ser Arg Leu Cys Ala Lys Gly Asp
    530                 535                 540
Ile Pro Pro Pro Leu Arg Ala Leu Pro Arg Tyr Arg Leu Leu Arg Asp
545                 550                 555                 560
Leu Pro Arg Leu Leu Arg Ala Leu Asp Ala Arg Pro Phe Ala Glu Ala
                565                 570                 575
Thr Ser Trp Gly Arg Leu Gly Ala Arg Gln Arg Arg Gln Ser Arg Leu
            580                 585                 590
Glu Leu Cys Ser Arg Leu Glu Arg Glu Ala Ala Arg Leu Ala Asp Leu
        595                 600                 605
Gly
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<210> 6
<211> 1827
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate nucleotide sequence encoding the amino
acid sequence of SEQ ID NO:5.

<221> misc_feature
<222> (1)...(1827)
<223> n = A, T, C or G

<400> 6
atgggnwsnw snmgnytngc ngcnytnytn ytnccnytny tnytnathgt nathgayytn 60
wsngaywsng cnggnathgg nttymgncay ytnccncayt ggaayacnmg ntgyccnytn 120
gcnwsncaya cngtnttyaa yggngcnwsn wsnacnwsnt ggtgymgnaa yccnaarwsn 180
ytnccncayw snwsnwsnat hggngayacn mgntgycarc ayytnytnmg nggnwsntgy 240
tgyytngtng tnacntgyyt nmgnmgngcn athacnttyc cnwsnccncc ncaracnwsn 300
ccnacnmgng ayttygcnyt naarggnccn aayytnmgna thcarmgnca yggnaargtn 360
ttyccngayt ggacncayaa rggnccngar ttywsnttyg ayytnytncc ngargcnmgn 420
gcnathmgng tnacnathws nwsnggnccn gargtnwsng tnmgnytntg ycaycartgg 480
gcnytngart gygargaryt nwsnwsnccn taygaygtnc araarathgt nwsnggnggn 540
cayacngtng arytnccnta ygarttyytn ytnccntgyy tntgyathga rgcnwsntay 600
ytncargarg ayacngtnmg nmgnaaraar tgyccnttyc arwsntggcc ngargcntay 660
ggnwsngayt tytggaarws ngtncaytty acngaytayw sncarcayac ncaratggtn 720
atggcnytna cnytnmgntg yccnytnaar ytngargcng cnytntgyca rmgncaygay 780
tggcayacny tntgyaarga yytnccnaay gcnacngcnm gngarwsnga yggntggtay 840
gtnytngara argtngayyt ncayccncar ytntgyttya arttywsntt yggnaaywsn 900
wsncaygtng artgyccnca ycaracnggn athacngarg cnmgngaytg gccnwsncay 960
athcargtnw sntgywsncc nggngtnccn athmgngarc cncaracnws naaytgyytn 1020
tggttygtnm gnaaygargc nacncarcar gargcnmgng gnwsnwsncc ngtnwsnytn 1080
gayytnatha thccnttyyt nmgnccnggn tgytgygtny tngtntggmg nwsngaygtn 1140
carttygcnt ggaarcayyt nytntgyccn gaygtnwsnt aymgncayyt nggnytnytn 1200
athytngcny tnytngcnyt nytnacnytn ytnggngtng tnytngcnyt nacntgymgn 1260
mgnccncarw snggnccngg nccngcnmgn ccngtnytny tnytncaygc ngcngaywsn 1320
gargcncarm gnmgnytngt nggngcnytn gcngarytny tnmgngcngc nytnggnggn 1380
ggnmgngayg tnathgtnga yytntgggar ggnmgncayg tngcnmgngt nggnccnytn 1440

ccntggytnt gggcngcnmg nacnmgngtn gcnmgngarc arggnacngt nytnytnytn 1500
tggwsnggng cngayytnmg nccngtnwsn ggnccngayc cnmgngcngc nccnytnytn 1560
gcnytnytnc aygcngcncc nmgnccnytn ytnytnytng cntayttyws nmgnytntgy 1620
gcnaarggng ayathccncc nccnytnmgn gcnytnccnm gntaymgnyt nytnmgngay 1680
ytnccnmgny tnytnmgngc nytngaygcn mgnccnttyg cngargcnac nwsntggggn 1740
mgnytnggng cnmgncarmg nmgncarwsn mgnytngary tntgywsnmg nytngarmgn 1800
gargcngcnm gnytngcnga yytnggn 1827

<210> 7
<211> 2245
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (66)...(1664)

<221> misc_feature
<222> (0)...(0)
<223> Zcytor21-f6

<400> 7
aggccctgcc acccaccttc aggccatgca gccatgttcc gggagcccta attgcacaga 60
agccc atg ggg agc tcc aga ctg gca gcc ctg ctc ctg cct ctc ctc ctc 110
Met Gly Ser Ser Arg Leu Ala Ala Leu Leu Leu Pro Leu Leu Leu
1 5 10 15

ata gtc atc gac ctc tct gac tct gct ggg att ggc ttt cgc cac ctg 158
Ile Val Ile Asp Leu Ser Asp Ser Ala Gly Ile Gly Phe Arg His Leu
20 25 30

ccc cac tgg aac acc cgc tgt cct ctg gcc tcc cac acg gat gac agt 206
Pro His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Asp Asp Ser
35 40 45

ttc act gga agt tct gcc tat atc cct tgc cgc acc tgg tgg gcc ctc 254
Phe Thr Gly Ser Ser Ala Tyr Ile Pro Cys Arg Thr Trp Trp Ala Leu
50 55 60

ttc tcc aca aag cct tgg tgt gtg cga gtc tgg cac tgt tcc cgc tgt 302
Phe Ser Thr Lys Pro Trp Cys Val Arg Val Trp His Cys Ser Arg Cys
65 70 75

ttg tgc cag cat ctg ctg tca ggt ggc tca ggt ctt caa cgg ggc ctc 350
Leu Cys Gln His Leu Leu Ser Gly Gly Ser Gly Leu Gln Arg Gly Leu
80 85 90 95

ttc cac ctc ctg gtg cag aaa tcc aaa aag tct tcc aca ttc aag ttc 398
Phe His Leu Leu Val Gln Lys Ser Lys Lys Ser Ser Thr Phe Lys Phe
100 105 110

tat agg aga cac aag atg cca gca cct gct cag agg aag ctg ctg cct 446
Tyr Arg Arg His Lys Met Pro Ala Pro Ala Gln Arg Lys Leu Leu Pro
115 120 125

cgt cgt cac ctg tct gag aag agc cat cac att tcc atc ccc tcc cca 494
Arg Arg His Leu Ser Glu Lys Ser His His Ile Ser Ile Pro Ser Pro
130 135 140

gac atc tcc cac aag gga ctt cgc tct aaa agg acc caa cct tcg gat 542
Asp Ile Ser His Lys Gly Leu Arg Ser Lys Arg Thr Gln Pro Ser Asp
145 150 155

```
cca gag aca tgg gaa agt ctt ccc aga ttg gac tca caa agg cat gga    590
Pro Glu Thr Trp Glu Ser Leu Pro Arg Leu Asp Ser Gln Arg His Gly
160                 165                 170                 175

gga ccc gag ttc tcc ttt gat ttg ctg cct gag gcc cgg gct att cgg    638
Gly Pro Glu Phe Ser Phe Asp Leu Leu Pro Glu Ala Arg Ala Ile Arg
                180                 185                 190

gtg acc ata tct tca ggc cct gag gtc agc gtg cgt ctt tgt cac cag    686
Val Thr Ile Ser Ser Gly Pro Glu Val Ser Val Arg Leu Cys His Gln
            195                 200                 205

tgg gca ctg gag tgt gaa gag ctg agc agt ccc tat gat gtc cag aaa    734
Trp Ala Leu Glu Cys Glu Glu Leu Ser Ser Pro Tyr Asp Val Gln Lys
        210                 215                 220

att gtg tct ggg ggc cac act gta gag ctg cct tat gaa ttc ctt ctg    782
Ile Val Ser Gly Gly His Thr Val Glu Leu Pro Tyr Glu Phe Leu Leu
    225                 230                 235

ccc tgt ctg tgc ata gag gca tcc tac ctg caa gag gac act gtg agg    830
Pro Cys Leu Cys Ile Glu Ala Ser Tyr Leu Gln Glu Asp Thr Val Arg
240                 245                 250                 255

cgc aaa aaa tgt ccc ttc cag agc tgg cca gaa gcc tat ggc tcg gac    878
Arg Lys Lys Cys Pro Phe Gln Ser Trp Pro Glu Ala Tyr Gly Ser Asp
                260                 265                 270

ttc tgg aag tca gtg cac ttc act gac tac agc cag cac act cag atg    926
Phe Trp Lys Ser Val His Phe Thr Asp Tyr Ser Gln His Thr Gln Met
            275                 280                 285

gtc atg gcc ctg aca ctc cgc tgc cca ctg aag ctg gaa gct gcc ctc    974
Val Met Ala Leu Thr Leu Arg Cys Pro Leu Lys Leu Glu Ala Ala Leu
        290                 295                 300

tgc cag agg cac gac tgg cat acc ctt tgc aaa gac ctc ccg aat gcc    1022
Cys Gln Arg His Asp Trp His Thr Leu Cys Lys Asp Leu Pro Asn Ala
    305                 310                 315

aca gct cga gag tca gat ggg tgg tat gtt ttg gag aag gtg gac ctg    1070
Thr Ala Arg Glu Ser Asp Gly Trp Tyr Val Leu Glu Lys Val Asp Leu
320                 325                 330                 335

cac ccc cag ctc tgc ttc aag ttc tct ttt gga aac agc agc cat gtt    1118
His Pro Gln Leu Cys Phe Lys Phe Ser Phe Gly Asn Ser Ser His Val
                340                 345                 350

gaa tgc ccc cac cag act ggg tct ctc aca tcc tgg aat gta agc atg    1166
Glu Cys Pro His Gln Thr Gly Ser Leu Thr Ser Trp Asn Val Ser Met
            355                 360                 365

gat acc caa gcc cag cag ctg att ctt cac ttc tcc tca aga atg cat    1214
Asp Thr Gln Ala Gln Gln Leu Ile Leu His Phe Ser Ser Arg Met His
        370                 375                 380

gcc acc ttc agt gct gcc tgg agc ctc cca ggc ttg ggg cag gac act    1262
Ala Thr Phe Ser Ala Ala Trp Ser Leu Pro Gly Leu Gly Gln Asp Thr
    385                 390                 395

ttg gtg ccc ccc gtg tac act gtc agc cag gcc cgg ggc tca agc cca    1310
```

```
Leu Val Pro Pro Val Tyr Thr Val Ser Gln Ala Arg Gly Ser Ser Pro
400                 405                 410                 415

gtg tca cta gac ctc atc att ccc ttc ctg agg cca ggg tgc tgt gtc    1358
Val Ser Leu Asp Leu Ile Ile Pro Phe Leu Arg Pro Gly Cys Cys Val
            420                 425                 430

ctg ctc cat gct tca ctc agc tcc ccg gga gga gaa gat gcc tgg ctc    1406
Leu Leu His Ala Ser Leu Ser Ser Pro Gly Gly Glu Asp Ala Trp Leu
        435                 440                 445

ata ggg gtg ggg ggc tct gtg ccc tca ggt gtg gcg gtc aga tgt cca    1454
Ile Gly Val Gly Gly Ser Val Pro Ser Gly Val Ala Val Arg Cys Pro
    450                 455                 460

gtt tgc ctg gaa gca cct ctt gtg tcc gga tgt ctc tta cag aca cct    1502
Val Cys Leu Glu Ala Pro Leu Val Ser Gly Cys Leu Leu Gln Thr Pro
465                 470                 475

ggg gct ctt gat cct ggc act gct ggc cct cct cac cct act ggg tgt    1550
Gly Ala Leu Asp Pro Gly Thr Ala Gly Pro Pro His Pro Thr Gly Cys
480                 485                 490                 495

tgt tct ggc cct cac ctg ccg gcg ccc aca gtc agg ccc ggg ccc agc    1598
Cys Ser Gly Pro His Leu Pro Ala Pro Thr Val Arg Pro Gly Pro Ser
                500                 505                 510

gcg gcc agt gct cct cct gca cgc ggc gga ctc gga ggc gca gcg gcg    1646
Ala Ala Ser Ala Pro Pro Ala Arg Gly Gly Leu Gly Gly Ala Ala Ala
            515                 520                 525

cct ggt ggg agc gct ggc tgaactgcta cgggcagcgc tgggcggcgg           1694
Pro Gly Gly Ser Ala Gly
        530

gcgcgacgtg atcgtggacc tgtgggaggg gaggcacgtg gcgcgcgtgg gcccgctgcc 1754
gtggctctgg gcggcgcgga cgcgcgtagc gcgggagcag ggcactgtgc tgctgctgtg 1814
gagcggcgcc gaccttcgcc cggtcagcgg ccccgacccc cgcgccgcgc ccctgctcgc 1874
cctgctccac gctgccccgc gcccgctgct gctgctcgct tacttcagtc gcctctgcgc 1934
caagggcgac atccccccgc cgctgcgcgc cctgccgcgc taccgcctgc tgcgcgacct 1994
gccgcgtctg ctgcgggcgc tggacgcgcg gcctttcgca gaggccacca gctggggccg 2054
ccttggggcg cggcagcgca ggcagagccg cctagagctg tgcagccggc tcgaacgaga 2114
ggccgcccga cttgcagacc taggttgagc agagctccac cgcagtcccg ggtgtctgcg 2174
gccgcaacgc aacggacact ggctggaacc ccggaatgag ccttcgaccc tgaaatcctt 2234
ggggtgcctc g                                                      2245

<210> 8
<211> 533
<212> PRT
<213> Homo sapiens

<400> 8
Met Gly Ser Ser Arg Leu Ala Ala Leu Leu Leu Pro Leu Leu Leu Ile
1               5                   10                  15
Val Ile Asp Leu Ser Asp Ser Ala Gly Ile Gly Phe Arg His Leu Pro
            20                  25                  30
His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Asp Asp Ser Phe
        35                  40                  45
Thr Gly Ser Ser Ala Tyr Ile Pro Cys Arg Thr Trp Trp Ala Leu Phe
    50                  55                  60
Ser Thr Lys Pro Trp Cys Val Arg Val Trp His Cys Ser Arg Cys Leu
65                  70                  75                  80
```

Cys Gln His Leu Leu Ser Gly Gly Ser Gly Leu Gln Arg Gly Leu Phe
85 90 95
His Leu Leu Val Gln Lys Ser Lys Lys Ser Ser Thr Phe Lys Phe Tyr
100 105 110
Arg Arg His Lys Met Pro Ala Pro Ala Gln Arg Lys Leu Leu Pro Arg
115 120 125
Arg His Leu Ser Glu Lys Ser His His Ile Ser Ile Pro Ser Pro Asp
130 135 140
Ile Ser His Lys Gly Leu Arg Ser Lys Arg Thr Gln Pro Ser Asp Pro
145 150 155 160
Glu Thr Trp Glu Ser Leu Pro Arg Leu Asp Ser Gln Arg His Gly Gly
165 170 175
Pro Glu Phe Ser Phe Asp Leu Leu Pro Glu Ala Arg Ala Ile Arg Val
180 185 190
Thr Ile Ser Ser Gly Pro Glu Val Ser Val Arg Leu Cys His Gln Trp
195 200 205
Ala Leu Glu Cys Glu Glu Leu Ser Ser Pro Tyr Asp Val Gln Lys Ile
210 215 220
Val Ser Gly Gly His Thr Val Glu Leu Pro Tyr Glu Phe Leu Leu Pro
225 230 235 240
Cys Leu Cys Ile Glu Ala Ser Tyr Leu Gln Glu Asp Thr Val Arg Arg
245 250 255
Lys Lys Cys Pro Phe Gln Ser Trp Pro Glu Ala Tyr Gly Ser Asp Phe
260 265 270
Trp Lys Ser Val His Phe Thr Asp Tyr Ser Gln His Thr Gln Met Val
275 280 285
Met Ala Leu Thr Leu Arg Cys Pro Leu Lys Leu Glu Ala Ala Leu Cys
290 295 300
Gln Arg His Asp Trp His Thr Leu Cys Lys Asp Leu Pro Asn Ala Thr
305 310 315 320
Ala Arg Glu Ser Asp Gly Trp Tyr Val Leu Glu Lys Val Asp Leu His
325 330 335
Pro Gln Leu Cys Phe Lys Phe Ser Phe Gly Asn Ser Ser His Val Glu
340 345 350
Cys Pro His Gln Thr Gly Ser Leu Thr Ser Trp Asn Val Ser Met Asp
355 360 365
Thr Gln Ala Gln Gln Leu Ile Leu His Phe Ser Ser Arg Met His Ala
370 375 380
Thr Phe Ser Ala Ala Trp Ser Leu Pro Gly Leu Gly Gln Asp Thr Leu
385 390 395 400
Val Pro Pro Val Tyr Thr Val Ser Gln Ala Arg Gly Ser Ser Pro Val
405 410 415
Ser Leu Asp Leu Ile Ile Pro Phe Leu Arg Pro Gly Cys Cys Val Leu
420 425 430
Leu His Ala Ser Leu Ser Ser Pro Gly Gly Glu Asp Ala Trp Leu Ile
435 440 445
Gly Val Gly Gly Ser Val Pro Ser Gly Val Ala Val Arg Cys Pro Val
450 455 460
Cys Leu Glu Ala Pro Leu Val Ser Gly Cys Leu Leu Gln Thr Pro Gly
465 470 475 480
Ala Leu Asp Pro Gly Thr Ala Gly Pro Pro His Pro Thr Gly Cys Cys
485 490 495
Ser Gly Pro His Leu Pro Ala Pro Thr Val Arg Pro Gly Pro Ser Ala
500 505 510
Ala Ser Ala Pro Pro Ala Arg Gly Gly Leu Gly Gly Ala Ala Ala Pro
515 520 525
Gly Gly Ser Ala Gly
530

<210> 9
<211> 1599
<212> DNA

<213> Artificial Sequence

<220>
<223> Degenerate nucleotide sequence encoding the amino
acid sequence of SEQ ID NO:8.

<221> misc_feature
<222> (1)...(1599)
<223> n = A, T, C or G

<400> 9
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atgggnwsnw snmgnytngc ngcnytnytn ytnccnytny tnytnathgt nathgayytn 60
wsngaywsng cnggnathgg nttymgncay ytnccncayt ggaayacnmg ntgyccnytn 120
gcnwsncaya cngaygayws nttyacnggn wsnwsngcnt ayathccntg ymgnacntgg 180
tgggcnytnt tywsnacnaa rccntggtgy gtnmgngtnt ggcaytgyws nmgntgyytn 240
tgycarcayy tnytnwsngg nggnwsnggn ytncarmgng gnytnttyca yytnytngtn 300
caraarwsna araarwsnws nacnttyaar ttytaymgnm gncayaarat gccngcnccn 360
gcncarmgna arytnytncc nmgnmgncay ytnwsngara arwsncayca yathwsnath 420
ccnwsnccng ayathwsnca yaarggnytn mgnwsnaarm gnacncarcc nwsngayccn 480
garacntggg arwsnytncc nmgnytngay wsncarmgnc ayggnggncc ngarttywsn 540
ttygayytny tnccngargc nmgngcnath mgngtnacna thwsnwsngg nccngargtn 600
wsngtnmgny tntgycayca rtgggcnytn gartgygarg arytnwsnws nccntaygay 660
gtncaraara thgtnwsngg nggncayacn gtngarytnc cntaygartt yytnytnccn 720
tgyytntgya thgargcnws ntayytncar gargayacng tnmgnmgnaa raartgyccn 780
ttycarwsnt ggccngargc ntayggnwsn gayttytgga arwsngtnca yttyacngay 840
taywsncarc ayacncarat ggtnatggcn ytnacnytnm gntgyccnyt naarytngar 900
gcngcnytnt gycarmgnca ygaytggcay acnytntgya argayytncc naaygcnacn 960
gcnmgngarw sngayggntg gtaygtnytn garaargtng ayytncaycc ncarytntgy 1020
ttyaarttyw snttyggnaa ywsnwsncay gtngartgyc cncaycarac nggnwsnytn 1080
acnwsntgga aygtnwsnat ggayacncar gcncarcary tnathytnca yttywsnwsn 1140
mgnatgcayg cnacnttyws ngcngcntgg wsnytnccng gnytnggnca rgayacnytn 1200
gtnccnccng tntayacngt nwsncargcn mgnggnwsnw snccngtnws nytngayytn 1260
athathccnt tyytnmgncc nggntgytgy gtnytnytnc aygcnwsnyt nwsnwsnccn 1320
ggnggngarg aygcntggyt nathggngtn ggnggnwsng tnccnwsngg ngtngcngtn 1380
mgntgyccng tntgyytnga rgcnccnytn gtnwsnggnt gyytnytnca racnccnggn 1440
gcnytngayc cnggnacngc nggnccnccn cayccnacng gntgytgyws nggnccncay 1500
ytnccngcnc cnacngtnmg nccnggnccn wsngcngcnw sngcnccncc ngcnmgnggn 1560
ggnytnggng gngcngcngc nccnggnggn wsngcnggn                         1599
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<210> 10
<211> 2172
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (66)...(2066)

<221> misc_feature
<222> (0)...(0)
<223> Zcytor21-d2

<400> 10
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aggccctgcc acccaccttc aggccatgca gccatgttcc gggagcccta attgcacaga 60
agccc atg ggg agc tcc aga ctg gca gcc ctg ctc ctg cct ctc ctc ctc 110
      Met Gly Ser Ser Arg Leu Ala Ala Leu Leu Leu Pro Leu Leu Leu
       1               5                  10                  15

ata gtc atc gac ctc tct gac tct gct ggg att ggc ttt cgc cac ctg   158
Ile Val Ile Asp Leu Ser Asp Ser Ala Gly Ile Gly Phe Arg His Leu
                20                  25                  30
```

```
ccc cac tgg aac acc cgc tgt cct ctg gcc tcc cac acg gat gac agt    206
Pro His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Asp Asp Ser
            35                  40                  45

ttc act gga agt tct gcc tat atc cct tgc cgc acc tgg tgg gcc ctc    254
Phe Thr Gly Ser Ser Ala Tyr Ile Pro Cys Arg Thr Trp Trp Ala Leu
        50                  55                  60

ttc tcc aca aag cct tgg tgt gtg cga gtc tgg cac tgt tcc cgc tgt    302
Phe Ser Thr Lys Pro Trp Cys Val Arg Val Trp His Cys Ser Arg Cys
    65                  70                  75

ttg tgc cag cat ctg ctg tca ggt ggc tca ggt ctt caa cgg ggc ctc    350
Leu Cys Gln His Leu Leu Ser Gly Gly Ser Gly Leu Gln Arg Gly Leu
80                  85                  90                  95

ttc cac ctc ctg gtg cag aaa tcc aaa aag tct tcc aca ttc aag ttc    398
Phe His Leu Leu Val Gln Lys Ser Lys Lys Ser Ser Thr Phe Lys Phe
                100                 105                 110

tat agg aga cac aag atg cca gca cct gct cag agg aag ctg ctg cct    446
Tyr Arg Arg His Lys Met Pro Ala Pro Ala Gln Arg Lys Leu Leu Pro
            115                 120                 125

cgt cgt cac ctg tct gag aag agc cat cac att tcc atc ccc tcc cca    494
Arg Arg His Leu Ser Glu Lys Ser His His Ile Ser Ile Pro Ser Pro
        130                 135                 140

gac atc tcc cac aag gga ctt cgc tct aaa agg acc caa cct tcg gat    542
Asp Ile Ser His Lys Gly Leu Arg Ser Lys Arg Thr Gln Pro Ser Asp
    145                 150                 155

cca gag aca tgg gaa agt ctt ccc aga ttg gac tca caa agg cat gga    590
Pro Glu Thr Trp Glu Ser Leu Pro Arg Leu Asp Ser Gln Arg His Gly
160                 165                 170                 175

gga ccc gag ttc tcc ttt gat ttg ctg cct gag gcc cgg gct att cgg    638
Gly Pro Glu Phe Ser Phe Asp Leu Leu Pro Glu Ala Arg Ala Ile Arg
                180                 185                 190

gtg acc ata tct tca ggc cct gag gtc agc gtg cgt ctt tgt cac cag    686
Val Thr Ile Ser Ser Gly Pro Glu Val Ser Val Arg Leu Cys His Gln
            195                 200                 205

tgg gca ctg gag tgt gaa gag ctg agc agt ccc tat gat gtc cag aaa    734
Trp Ala Leu Glu Cys Glu Glu Leu Ser Ser Pro Tyr Asp Val Gln Lys
        210                 215                 220

att gtg tct ggg ggc cac act gta gag ctg cct tat gaa ttc ctt ctg    782
Ile Val Ser Gly Gly His Thr Val Glu Leu Pro Tyr Glu Phe Leu Leu
    225                 230                 235

ccc tgt ctg tgc ata gag gca tcc tac ctg caa gag gac act gtg agg    830
Pro Cys Leu Cys Ile Glu Ala Ser Tyr Leu Gln Glu Asp Thr Val Arg
240                 245                 250                 255

cgc aaa aaa tgt ccc ttc cag agc tgg cca gaa gcc tat ggc tcg gac    878
Arg Lys Lys Cys Pro Phe Gln Ser Trp Pro Glu Ala Tyr Gly Ser Asp
                260                 265                 270

ttc tgg aag tca gtg cac ttc act gac tac agc cag cac act cag atg    926
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Phe Trp Lys Ser Val His Phe Thr Asp Tyr Ser Gln His Thr Gln Met
275 280 285

gtc atg gcc ctg aca ctc cgc tgc cca ctg aag ctg gaa gct gcc ctc 974
Val Met Ala Leu Thr Leu Arg Cys Pro Leu Lys Leu Glu Ala Ala Leu
290 295 300

tgc cag agg cac gac tgg cat acc ctt tgc aaa gac ctc ccg aat gcc 1022
Cys Gln Arg His Asp Trp His Thr Leu Cys Lys Asp Leu Pro Asn Ala
305 310 315

acg gct cga gag tca gat ggg tgg tat gtt ttg gag aag gtg gac ctg 1070
Thr Ala Arg Glu Ser Asp Gly Trp Tyr Val Leu Glu Lys Val Asp Leu
320 325 330 335

cac ccc cag ctc tgc ttc aag ttc tct ttt gga aac agc agc cat gtt 1118
His Pro Gln Leu Cys Phe Lys Phe Ser Phe Gly Asn Ser Ser His Val
340 345 350

gaa tgc ccc cac cag act ggg tct ctc aca tcc tgg aat gta agc atg 1166
Glu Cys Pro His Gln Thr Gly Ser Leu Thr Ser Trp Asn Val Ser Met
355 360 365

gat acc caa gcc cag cag ctg att ctt cac ttc tcc tca aga atg cat 1214
Asp Thr Gln Ala Gln Gln Leu Ile Leu His Phe Ser Ser Arg Met His
370 375 380

gcc acc ttc agt gct gcc tgg agc ctc cca ggc ttg ggg cag gac act 1262
Ala Thr Phe Ser Ala Ala Trp Ser Leu Pro Gly Leu Gly Gln Asp Thr
385 390 395

ttg gtg ccc ccc gtg tac act gtc agc cag gcc cgg ggc tca agc cca 1310
Leu Val Pro Pro Val Tyr Thr Val Ser Gln Ala Arg Gly Ser Ser Pro
400 405 410 415

gtg tca cta gac ctc atc att ccc ttc ctg agg cca ggg tgc tgt gtc 1358
Val Ser Leu Asp Leu Ile Ile Pro Phe Leu Arg Pro Gly Cys Cys Val
420 425 430

ctg gtg tgg cgg tca gat gtc cag ttt gcc tgg aag cac ctc ttg tgt 1406
Leu Val Trp Arg Ser Asp Val Gln Phe Ala Trp Lys His Leu Leu Cys
435 440 445

cca gat gtc tct tac aga cac ctg ggg ctc ttg atc ctg gca ctg ctg 1454
Pro Asp Val Ser Tyr Arg His Leu Gly Leu Leu Ile Leu Ala Leu Leu
450 455 460

gcc ctc ctc acc cta ctg ggt gtt gtt ctg gcc ctc acc tgc cgg cgc 1502
Ala Leu Leu Thr Leu Leu Gly Val Val Leu Ala Leu Thr Cys Arg Arg
465 470 475

cca cag tca ggc ccg ggc cca gcg cgg cca gtg ctc ctc ctg cac gcg 1550
Pro Gln Ser Gly Pro Gly Pro Ala Arg Pro Val Leu Leu Leu His Ala
480 485 490 495

gcg gac tcg gag gcg cag cgg cgc ctg gtg gga gcg ctg gct gaa ctg 1598
Ala Asp Ser Glu Ala Gln Arg Arg Leu Val Gly Ala Leu Ala Glu Leu
500 505 510

cta cgg gca gcg ctg ggc ggc ggg cgc gac gtg atc gtg gac ctg tgg 1646

Leu Arg Ala Ala Leu Gly Gly Gly Arg Asp Val Ile Val Asp Leu Trp
515 520 525

gag ggg agg cac gtg gcg cgc gtg ggc ccg ctg ccg tgg ctc tgg gcg 1694
Glu Gly Arg His Val Ala Arg Val Gly Pro Leu Pro Trp Leu Trp Ala
530 535 540

gcg cgg acg cgc gta gcg cgg gag cag ggc act gtg ctg ctg ctg tgg 1742
Ala Arg Thr Arg Val Ala Arg Glu Gln Gly Thr Val Leu Leu Leu Trp
545 550 555

agc ggc gcc gac ctt cgc ccg gtc agc ggc ccc gac ccc cgc gcc gcg 1790
Ser Gly Ala Asp Leu Arg Pro Val Ser Gly Pro Asp Pro Arg Ala Ala
560 565 570 575

ccc ctg ctc gcc ctg ctc cac gct gcc ccg cgc ccg ctg ctg ctg ctc 1838
Pro Leu Leu Ala Leu Leu His Ala Ala Pro Arg Pro Leu Leu Leu Leu
580 585 590

gct tac ttc agt cgc ctc tgc gcc aag ggc gac atc ccc ccg ccg ctg 1886
Ala Tyr Phe Ser Arg Leu Cys Ala Lys Gly Asp Ile Pro Pro Pro Leu
595 600 605

cgc gcc ctg ccg cgc tac cgc ctg ctg cgc gac ctg ccg cgt ctg ctg 1934
Arg Ala Leu Pro Arg Tyr Arg Leu Leu Arg Asp Leu Pro Arg Leu Leu
610 615 620

cgg gcg ctg gac gcg cgg cct ttc gca gag gcc acc agc tgg ggc cgc 1982
Arg Ala Leu Asp Ala Arg Pro Phe Ala Glu Ala Thr Ser Trp Gly Arg
625 630 635

ctt ggg gcg cgg cag cgc agg cag agc cgc cta gag ctg tgc agc cgg 2030
Leu Gly Ala Arg Gln Arg Arg Gln Ser Arg Leu Glu Leu Cys Ser Arg
640 645 650 655

ctt gaa cga gag gcc gcc cga ctt gca gac cta ggt tgagcagagc 2076
Leu Glu Arg Glu Ala Ala Arg Leu Ala Asp Leu Gly
660 665

tccaccgcag tcccgggtgt ctgcggccgc aacgcaacgg acactggctg gaaccccgga 2136
atgagccttc gaccctgaaa tccttggggt gcctcg 2172

<210> 11
<211> 667
<212> PRT
<213> Homo sapiens

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Val Ile Asp Leu Ser Asp Ser Ala Gly Ile Gly Phe Arg His Leu Pro
20 25 30
His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Asp Asp Ser Phe
35 40 45
Thr Gly Ser Ser Ala Tyr Ile Pro Cys Arg Thr Trp Trp Ala Leu Phe
50 55 60
Ser Thr Lys Pro Trp Cys Val Arg Val Trp His Cys Ser Arg Cys Leu
65 70 75 80
Cys Gln His Leu Leu Ser Gly Gly Ser Gly Leu Gln Arg Gly Leu Phe
85 90 95

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His Leu Leu Val Gln Lys Ser Lys Lys Ser Ser Thr Phe Lys Phe Tyr
                100                 105                 110
Arg Arg His Lys Met Pro Ala Pro Ala Gln Arg Lys Leu Leu Pro Arg
        115                 120                 125
Arg His Leu Ser Glu Lys Ser His His Ile Ser Ile Pro Ser Pro Asp
    130                 135                 140
Ile Ser His Lys Gly Leu Arg Ser Lys Arg Thr Gln Pro Ser Asp Pro
145                 150                 155                 160
Glu Thr Trp Glu Ser Leu Pro Arg Leu Asp Ser Gln Arg His Gly Gly
                165                 170                 175
Pro Glu Phe Ser Phe Asp Leu Leu Pro Glu Ala Arg Ala Ile Arg Val
            180                 185                 190
Thr Ile Ser Ser Gly Pro Glu Val Ser Val Arg Leu Cys His Gln Trp
        195                 200                 205
Ala Leu Glu Cys Glu Glu Leu Ser Ser Pro Tyr Asp Val Gln Lys Ile
    210                 215                 220
Val Ser Gly Gly His Thr Val Glu Leu Pro Tyr Glu Phe Leu Leu Pro
225                 230                 235                 240
Cys Leu Cys Ile Glu Ala Ser Tyr Leu Gln Glu Asp Thr Val Arg Arg
                245                 250                 255
Lys Lys Cys Pro Phe Gln Ser Trp Pro Glu Ala Tyr Gly Ser Asp Phe
            260                 265                 270
Trp Lys Ser Val His Phe Thr Asp Tyr Ser Gln His Thr Gln Met Val
        275                 280                 285
Met Ala Leu Thr Leu Arg Cys Pro Leu Lys Leu Glu Ala Ala Leu Cys
    290                 295                 300
Gln Arg His Asp Trp His Thr Leu Cys Lys Asp Leu Pro Asn Ala Thr
305                 310                 315                 320
Ala Arg Glu Ser Asp Gly Trp Tyr Val Leu Glu Lys Val Asp Leu His
                325                 330                 335
Pro Gln Leu Cys Phe Lys Phe Ser Phe Gly Asn Ser Ser His Val Glu
            340                 345                 350
Cys Pro His Gln Thr Gly Ser Leu Thr Ser Trp Asn Val Ser Met Asp
        355                 360                 365
Thr Gln Ala Gln Gln Leu Ile Leu His Phe Ser Ser Arg Met His Ala
    370                 375                 380
Thr Phe Ser Ala Ala Trp Ser Leu Pro Gly Leu Gly Gln Asp Thr Leu
385                 390                 395                 400
Val Pro Pro Val Tyr Thr Val Ser Gln Ala Arg Gly Ser Ser Pro Val
                405                 410                 415
Ser Leu Asp Leu Ile Ile Pro Phe Leu Arg Pro Gly Cys Cys Val Leu
            420                 425                 430
Val Trp Arg Ser Asp Val Gln Phe Ala Trp Lys His Leu Leu Cys Pro
        435                 440                 445
Asp Val Ser Tyr Arg His Leu Gly Leu Leu Ile Leu Ala Leu Leu Ala
    450                 455                 460
Leu Leu Thr Leu Leu Gly Val Val Leu Ala Leu Thr Cys Arg Arg Pro
465                 470                 475                 480
Gln Ser Gly Pro Gly Pro Ala Arg Pro Val Leu Leu Leu His Ala Ala
                485                 490                 495
Asp Ser Glu Ala Gln Arg Arg Leu Val Gly Ala Leu Ala Glu Leu Leu
            500                 505                 510
Arg Ala Ala Leu Gly Gly Gly Arg Asp Val Ile Val Asp Leu Trp Glu
        515                 520                 525
Gly Arg His Val Ala Arg Val Gly Pro Leu Pro Trp Leu Trp Ala Ala
    530                 535                 540
Arg Thr Arg Val Ala Arg Glu Gln Gly Thr Val Leu Leu Leu Trp Ser
545                 550                 555                 560
Gly Ala Asp Leu Arg Pro Val Ser Gly Pro Asp Pro Arg Ala Ala Pro
                565                 570                 575
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Leu Leu Ala Leu Leu His Ala Ala Pro Arg Pro Leu Leu Leu Leu Ala
580 585 590
Tyr Phe Ser Arg Leu Cys Ala Lys Gly Asp Ile Pro Pro Pro Leu Arg
595 600 605
Ala Leu Pro Arg Tyr Arg Leu Leu Arg Asp Leu Pro Arg Leu Leu Arg
610 615 620
Ala Leu Asp Ala Arg Pro Phe Ala Glu Ala Thr Ser Trp Gly Arg Leu
625 630 635 640
Gly Ala Arg Gln Arg Arg Gln Ser Arg Leu Glu Leu Cys Ser Arg Leu
645 650 655
Glu Arg Glu Ala Ala Arg Leu Ala Asp Leu Gly
660 665

<210> 12
<211> 2001
<212> DNA
<213> Artificial Sequence

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<223> Degenerate nucleotide sequence encoding the amino acid sequence of SEQ ID NO:11.

<221> misc_feature
<222> (1)...(2001)
<223> n = A, T, C or G

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atgggnwsnw snmgnytngc ngcnytnytn ytnccnytny tnytnathgt nathgayytn 60
wsngaywsng cnggnathgg nttymgncay ytnccncayt ggaayacnmg ntgyccnytn 120
gcnwsncaya cngaygayws nttyacnggn wsnwsngcnt ayathccntg ymgnacntgg 180
tgggcnytnt tywsnacnaa rccntggtgy gtnmgngtnt ggcaytgyws nmgntgyytn 240
tgycarcayy tnytnwsngg nggnwsnggn ytncarmgng gnytnttyca yytnytngtn 300
caraarwsna araarwsnws nacnttyaar ttytaymgnm gncayaarat gccngcnccn 360
gcncarmgna arytnytncc nmgnmgncay ytnwsngara arwsncayca yathwsnath 420
ccnwsnccng ayathwsnca yaarggnytn mgnwsnaarm gnacncarcc nwsngayccn 480
garacntggg arwsnytncc nmgnytngay wsncarmgnc ayggnggncc ngarttywsn 540
ttygayytny tnccngargc nmgngcnath mgngtnacna thwsnwsngg nccngargtn 600
wsngtnmgny tntgycayca rtgggcnytn gartgygarg arytnwsnws nccntaygay 660
gtncaraara thgtnwsngg nggncayacn gtngarytnc cntaygartt yytnytnccn 720
tgyytntgya thgargcnws ntayytncar gargayacng tnmgnmgnaa raartgyccn 780
ttycarwsnt ggccngargc ntayggnwsn gayttytgga arwsngtnca yttyacngay 840
taywsncarc ayacncarat ggtnatggcn ytnacnytnm gntgyccnyt naarytngar 900
gcngcnytnt gycarmgnca ygaytggcay acnytntgya argayytncc naaygcnacn 960
gcnmgngarw sngayggntg gtaygtnytn garaargtng ayytncaycc ncarytntgy 1020
ttyaarttyw snttyggnaa ywsnwsncay gtngartgyc cncaycarac nggnwsnytn 1080
acnwsntgga aygtnwsnat ggayacncar gcncarcary tnathytnca yttywsnwsn 1140
mgnatgcayg cnacnttyws ngcngcntgg wsnytnccng gnytnggnca rgayacnytn 1200
gtnccnccng tntayacngt nwsncargcn mgnggnwsnw snccngtnws nytngayytn 1260
athathccnt tyytnmgncc nggntgytgy gtnytngtnt ggmgnwsnga ygtncartty 1320
gcntggaarc ayytnytntg yccngaygtn wsntaymgnc ayytnggnyt nytnathytn 1380
gcnytnytng cnytnytnac nytnytnggn gtngtnytng cnytnacntg ymgnmgnccn 1440
carwsnggnc cnggnccngc nmgnccngtn ytnytnytnc aygcngcnga ywsngargcn 1500
carmgnmgny tngtnggngc nytngcngar ytnytnmgng cngcnytngg nggnggnmgn 1560
gaygtnathg tngayytntg ggarggnmgn caygtngcnm gngtnggncc nytnccntgg 1620
ytntgggcng cnmgnacnmg ngtngcnmgn garcarggna cngtnytnyt nytntggwsn 1680
ggngcngayy tnmgnccngt nwsnggnccn gayccnmgng cngcnccnyt nytngcnytn 1740
ytncaygcng cnccnmgncc nytnytnytn ytngcntayt tywsnmgnyt ntgygcnaar 1800
ggngayathc cnccnccnyt nmgngcnytn ccnmgntaym gnytnytnmg ngayytnccn 1860
mgnytnytnm gngcnytnga ygcnmgnccn ttygcngarg cnacnwsntg gggnmgnytn 1920
ggngcnmgnc armgnmgnca rwsnmgnytn garytntgyw snmgnytnga rmgngargcn 1980
gcnmgnytng cngayytngg n 2001

<210> 13
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide linker

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Gly Gly Ser Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 1   5   10   15

<210> 14
<211> 1974
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(1971)

<221> misc_feature
<222> (0)...(0)
<223> Zcytor21-g13

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Met Gly Ser Ser Arg Leu Ala Ala Leu Leu Leu Pro Leu Leu Leu Ile
 1   5   10   15

gtc atc gac ctc tct gac tct gct ggg att ggc ttt cgc cac ctg ccc   96
Val Ile Asp Leu Ser Asp Ser Ala Gly Ile Gly Phe Arg His Leu Pro
            20   25   30

cac tgg aac acc cgc tgt cct ctg gcc tcc cac acg gaa gtt ctg cct   144
His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Glu Val Leu Pro
        35   40   45

ata tcc ctt gcc gca cct ggt ggg ccc tct tct cca caa agc ctt ggt   192
Ile Ser Leu Ala Ala Pro Gly Gly Pro Ser Ser Pro Gln Ser Leu Gly
    50   55   60

gtg tgc gag tct ggc act gtt ccc gct gtt tgt gcc agc atc tgc tgt   240
Val Cys Glu Ser Gly Thr Val Pro Ala Val Cys Ala Ser Ile Cys Cys
65   70   75   80

cag gtg gct cag gtc ttc aac ggg gcc tct tcc acc tcc tgg tgc aga   288
Gln Val Ala Gln Val Phe Asn Gly Ala Ser Ser Thr Ser Trp Cys Arg
                85   90   95

aat cca aaa agt ctt cca cat tca agt tct ata gga gac aca aga tgc   336
Asn Pro Lys Ser Leu Pro His Ser Ser Ser Ile Gly Asp Thr Arg Cys
            100   105   110

cag cac ctg ctc aga gga agc tgc tgc ctc gtc gtc acc tgt ctg aga   384
Gln His Leu Leu Arg Gly Ser Cys Cys Leu Val Val Thr Cys Leu Arg
        115   120   125

aga gcc atc aca ttt cca tcc cct ccc cag aca tct ccc aca agg gac   432
Arg Ala Ile Thr Phe Pro Ser Pro Pro Gln Thr Ser Pro Thr Arg Asp
    130   135   140

ttc gct cta aaa gga ccc aac ctt cgg atc cag aga cat ggg aaa gtc 480
Phe Ala Leu Lys Gly Pro Asn Leu Arg Ile Gln Arg His Gly Lys Val
145 150 155 160

ttc cca gat tgg act cac aaa ggc atg gag gtg ggc act ggg tac aac 528
Phe Pro Asp Trp Thr His Lys Gly Met Glu Val Gly Thr Gly Tyr Asn
165 170 175

agg aga tgg gtt cag ctg agt ggt gga ccc gag ttc tcc ttt gat ttg 576
Arg Arg Trp Val Gln Leu Ser Gly Gly Pro Glu Phe Ser Phe Asp Leu
180 185 190

ctg cct gag gcc cgg gct att cgg gtg acc ata tct tca ggc cct gag 624
Leu Pro Glu Ala Arg Ala Ile Arg Val Thr Ile Ser Ser Gly Pro Glu
195 200 205

gtc agc gtg cgt ctt tgt cac cag tgg gca ctg gag tgt gaa gag ctg 672
Val Ser Val Arg Leu Cys His Gln Trp Ala Leu Glu Cys Glu Glu Leu
210 215 220

agc agt ccc tat gat gtc cag aaa att gtg tct ggg ggc cac act gta 720
Ser Ser Pro Tyr Asp Val Gln Lys Ile Val Ser Gly Gly His Thr Val
225 230 235 240

gag ctg cct tat gaa ttc ctt ctg ccc tgt ctg tgc ata gag gca tcc 768
Glu Leu Pro Tyr Glu Phe Leu Leu Pro Cys Leu Cys Ile Glu Ala Ser
245 250 255

tac ctg caa gag gac act gtg agg cgc aaa aaa tgt ccc ttc cag agc 816
Tyr Leu Gln Glu Asp Thr Val Arg Arg Lys Lys Cys Pro Phe Gln Ser
260 265 270

tgg cca gaa gcc tat ggc tcg gac ttc tgg aag tca gtg cac ttc act 864
Trp Pro Glu Ala Tyr Gly Ser Asp Phe Trp Lys Ser Val His Phe Thr
275 280 285

gac tac agc cag cac act cag atg gtc atg gcc ctg aca ctc cgc tgc 912
Asp Tyr Ser Gln His Thr Gln Met Val Met Ala Leu Thr Leu Arg Cys
290 295 300

cca ctg aag ctg gaa gct gcc ctc tgc cag agg cac gac tgg cat acc 960
Pro Leu Lys Leu Glu Ala Ala Leu Cys Gln Arg His Asp Trp His Thr
305 310 315 320

ctt tgc aaa gac ctc ccg aat gcc acg gct cga gag tca gat ggg tgg 1008
Leu Cys Lys Asp Leu Pro Asn Ala Thr Ala Arg Glu Ser Asp Gly Trp
325 330 335

tat gtt ttg gag aag gtg gac ctg cac ccc cag ctc tgc ttc aag gta 1056
Tyr Val Leu Glu Lys Val Asp Leu His Pro Gln Leu Cys Phe Lys Val
340 345 350

caa cca tgg ttc tct ttt gga aac agc agc cat gtt gaa tgc ccc cac 1104
Gln Pro Trp Phe Ser Phe Gly Asn Ser Ser His Val Glu Cys Pro His
355 360 365

cag act ggg tct ctc aca tcc tgg aat gta agc atg gat acc caa gcc 1152
Gln Thr Gly Ser Leu Thr Ser Trp Asn Val Ser Met Asp Thr Gln Ala
370 375 380

cag cag ctg att ctt cac ttc tcc tca aga atg cat gcc acc ttc agt 1200

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Gln Gln Leu Ile Leu His Phe Ser Ser Arg Met His Ala Thr Phe Ser
385                 390                 395                 400

gct gcc tgg agc ctc cca ggc ttg ggg cag gac act ttg gtg ccc ccc      1248
Ala Ala Trp Ser Leu Pro Gly Leu Gly Gln Asp Thr Leu Val Pro Pro
                405                 410                 415

gtg tac act gtc agc cag gtg tgg cgg tca gat gtc cag ttt gcc tgg      1296
Val Tyr Thr Val Ser Gln Val Trp Arg Ser Asp Val Gln Phe Ala Trp
            420                 425                 430

aag cac ctc ttg tgt cca gat gtc tct tac aga cac ctg ggg ctc ttg      1344
Lys His Leu Leu Cys Pro Asp Val Ser Tyr Arg His Leu Gly Leu Leu
        435                 440                 445

atc ctg gca ctg ctg gcc ctc ctc acc cta ctg ggt gtt gtt ctg gcc      1392
Ile Leu Ala Leu Leu Ala Leu Leu Thr Leu Leu Gly Val Val Leu Ala
    450                 455                 460

ctc acc tgc cgg cgc cca cag tca ggc ccg ggc cca gcg cgg cca gtg      1440
Leu Thr Cys Arg Arg Pro Gln Ser Gly Pro Gly Pro Ala Arg Pro Val
465                 470                 475                 480

ctc ctc ctg cac gcg gcg gac tcg gag gcg cag cgg cgc ctg gtg gga      1488
Leu Leu Leu His Ala Ala Asp Ser Glu Ala Gln Arg Arg Leu Val Gly
                485                 490                 495

gcg ctg gct gaa ctg cta cgg gca gcg ctg ggc ggc ggg cgc gac gtg      1536
Ala Leu Ala Glu Leu Leu Arg Ala Ala Leu Gly Gly Gly Arg Asp Val
            500                 505                 510

atc gtg gac ctg tgg gag ggg agg cac gtg gcg cgc gtg ggc ccg ctg      1584
Ile Val Asp Leu Trp Glu Gly Arg His Val Ala Arg Val Gly Pro Leu
        515                 520                 525

ccg tgg ctc tgg gcg gcg cgg acg cgc gta gcg cgg gag cag ggc act      1632
Pro Trp Leu Trp Ala Ala Arg Thr Arg Val Ala Arg Glu Gln Gly Thr
    530                 535                 540

gtg ctg ctg ctg tgg agc ggc gcc gac ctt cgc ccg gtc agc ggc ccc      1680
Val Leu Leu Leu Trp Ser Gly Ala Asp Leu Arg Pro Val Ser Gly Pro
545                 550                 555                 560

gac ccc cgc gcc gcg ccc ctg ctc gcc ctg ctc cac gct gcc ccg cgc      1728
Asp Pro Arg Ala Ala Pro Leu Leu Ala Leu Leu His Ala Ala Pro Arg
                565                 570                 575

ccg ctg ctg ctg ctc gct tac ttc agt cgc ctc tgc gcc aag ggc gac      1776
Pro Leu Leu Leu Leu Ala Tyr Phe Ser Arg Leu Cys Ala Lys Gly Asp
            580                 585                 590

atc ccc ccg ccg ctg cgc gcc ctg ccg cgc tac cgc ctg ctg cgc gac      1824
Ile Pro Pro Pro Leu Arg Ala Leu Pro Arg Tyr Arg Leu Leu Arg Asp
        595                 600                 605

ctg ccg cgt ctg ctg cgg gcg ctg gac gcg cgg cct ttc gca gag gcc      1872
Leu Pro Arg Leu Leu Arg Ala Leu Asp Ala Arg Pro Phe Ala Glu Ala
    610                 615                 620

acc agc tgg ggc cgc ctt ggg gcg cgg cag cgc agg cag agc cgc cta      1920
Thr Ser Trp Gly Arg Leu Gly Ala Arg Gln Arg Arg Gln Ser Arg Leu
625                 630                 635                 640
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gag ctg tgc agc cgg ctt gaa cga gag gcc gcc cga ctt gca gac cta 1968
Glu Leu Cys Ser Arg Leu Glu Arg Glu Ala Ala Arg Leu Ala Asp Leu
645 650 655

ggt tga 1974
Gly

<210> 15
<211> 657
<212> PRT
<213> Homo sapiens

<400> 15
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1 5 10 15
Val Ile Asp Leu Ser Asp Ser Ala Gly Ile Gly Phe Arg His Leu Pro
20 25 30
His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Glu Val Leu Pro
35 40 45
Ile Ser Leu Ala Ala Pro Gly Gly Pro Ser Ser Pro Gln Ser Leu Gly
50 55 60
Val Cys Glu Ser Gly Thr Val Pro Ala Val Cys Ala Ser Ile Cys Cys
65 70 75 80
Gln Val Ala Gln Val Phe Asn Gly Ala Ser Ser Thr Ser Trp Cys Arg
85 90 95
Asn Pro Lys Ser Leu Pro His Ser Ser Ser Ile Gly Asp Thr Arg Cys
100 105 110
Gln His Leu Leu Arg Gly Ser Cys Cys Leu Val Val Thr Cys Leu Arg
115 120 125
Arg Ala Ile Thr Phe Pro Ser Pro Pro Gln Thr Ser Pro Thr Arg Asp
130 135 140
Phe Ala Leu Lys Gly Pro Asn Leu Arg Ile Gln Arg His Gly Lys Val
145 150 155 160
Phe Pro Asp Trp Thr His Lys Gly Met Glu Val Gly Thr Gly Tyr Asn
165 170 175
Arg Arg Trp Val Gln Leu Ser Gly Gly Pro Glu Phe Ser Phe Asp Leu
180 185 190
Leu Pro Glu Ala Arg Ala Ile Arg Val Thr Ile Ser Ser Gly Pro Glu
195 200 205
Val Ser Val Arg Leu Cys His Gln Trp Ala Leu Glu Cys Glu Glu Leu
210 215 220
Ser Ser Pro Tyr Asp Val Gln Lys Ile Val Ser Gly Gly His Thr Val
225 230 235 240
Glu Leu Pro Tyr Glu Phe Leu Leu Pro Cys Leu Cys Ile Glu Ala Ser
245 250 255
Tyr Leu Gln Glu Asp Thr Val Arg Arg Lys Lys Cys Pro Phe Gln Ser
260 265 270
Trp Pro Glu Ala Tyr Gly Ser Asp Phe Trp Lys Ser Val His Phe Thr
275 280 285
Asp Tyr Ser Gln His Thr Gln Met Val Met Ala Leu Thr Leu Arg Cys
290 295 300
Pro Leu Lys Leu Glu Ala Ala Leu Cys Gln Arg His Asp Trp His Thr
305 310 315 320
Leu Cys Lys Asp Leu Pro Asn Ala Thr Ala Arg Glu Ser Asp Gly Trp
325 330 335
Tyr Val Leu Glu Lys Val Asp Leu His Pro Gln Leu Cys Phe Lys Val
340 345 350
Gln Pro Trp Phe Ser Phe Gly Asn Ser Ser His Val Glu Cys Pro His
355 360 365
Gln Thr Gly Ser Leu Thr Ser Trp Asn Val Ser Met Asp Thr Gln Ala
370 375 380

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Gln Gln Leu Ile Leu His Phe Ser Ser Arg Met His Ala Thr Phe Ser
385                 390                 395                 400
Ala Ala Trp Ser Leu Pro Gly Leu Gly Gln Asp Thr Leu Val Pro Pro
                405                 410                 415
Val Tyr Thr Val Ser Gln Val Trp Arg Ser Asp Val Gln Phe Ala Trp
            420                 425                 430
Lys His Leu Leu Cys Pro Asp Val Ser Tyr Arg His Leu Gly Leu Leu
        435                 440                 445
Ile Leu Ala Leu Leu Ala Leu Leu Thr Leu Leu Gly Val Val Leu Ala
    450                 455                 460
Leu Thr Cys Arg Arg Pro Gln Ser Gly Pro Gly Pro Ala Arg Pro Val
465                 470                 475                 480
Leu Leu Leu His Ala Ala Asp Ser Glu Ala Gln Arg Arg Leu Val Gly
                485                 490                 495
Ala Leu Ala Glu Leu Leu Arg Ala Ala Leu Gly Gly Gly Arg Asp Val
            500                 505                 510
Ile Val Asp Leu Trp Glu Gly Arg His Val Ala Arg Val Gly Pro Leu
        515                 520                 525
Pro Trp Leu Trp Ala Ala Arg Thr Arg Val Ala Arg Glu Gln Gly Thr
    530                 535                 540
Val Leu Leu Leu Trp Ser Gly Ala Asp Leu Arg Pro Val Ser Gly Pro
545                 550                 555                 560
Asp Pro Arg Ala Ala Pro Leu Leu Ala Leu Leu His Ala Ala Pro Arg
                565                 570                 575
Pro Leu Leu Leu Leu Ala Tyr Phe Ser Arg Leu Cys Ala Lys Gly Asp
            580                 585                 590
Ile Pro Pro Pro Leu Arg Ala Leu Pro Arg Tyr Arg Leu Leu Arg Asp
        595                 600                 605
Leu Pro Arg Leu Leu Arg Ala Leu Asp Ala Arg Pro Phe Ala Glu Ala
    610                 615                 620
Thr Ser Trp Gly Arg Leu Gly Ala Arg Gln Arg Arg Gln Ser Arg Leu
625                 630                 635                 640
Glu Leu Cys Ser Arg Leu Glu Arg Glu Ala Ala Arg Leu Ala Asp Leu
                645                 650                 655
Gly
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<210> 16
<211> 1971
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate nucleotide sequence encoding the amino
      acid sequence of SEQ ID NO:15.

<221> misc_feature
<222> (1)...(1971)
<223> n = A, T, C or G

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gcnwsncaya cngargtnyt nccnathwsn ytngcngcnc cnggnggncc nwsnwsnccn 180
carwsnytng gngtntgyga rwsnggnacn gtnccngcng tntgygcnws nathtgytgy 240
cargtngcnc argtnttyaa yggngcnwsn wsnacnwsnt ggtgymgnaa yccnaarwsn 300
ytnccncayw snwsnwsnat hggngayacn mgntgycarc ayytnytnmg nggnwsntgy 360
tgyytngtng tnacntgyyt nmgnmgngcn athacnttyc cnwsnccncc ncaracnwsn 420
ccnacnmgng ayttygcnyt naarggnccn aayytnmgna thcarmgnca yggnaargtn 480
ttyccngayt ggacncayaa rggnatggar gtnggnacng gntayaaymg nmgntgggtn 540
carytnwsng gnggnccnga rttywsntty gayytnytnc cngargcnmg ngcnathmgn 600
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gtnacnathw snwsnggncc ngargtnwsn gtnmgnytnt gycaycartg ggcnytngar 660
tgygargary tnwsnwsncc ntaygaygtn caraarathg tnwsnggngg ncayacngtn 720
garytnccnt aygarttyyt nytnccntgy ytntgyathg argcnwsnta yytncargar 780
gayacngtnm gnmgnaaraa rtgyccntty carwsntggc cngargcnta yggnwsngay 840
ttytggaarw sngtncaytt yacngaytay wsncarcaya cncaratggt natggcnytn 900
acnytnmgnt gyccnytnaa rytngargcn gcnytntgyc armgncayga ytggcayacn 960
ytntgyaarg ayytnccnaa ygcnacngcn mgngarwsng ayggntggta ygtnytngar 1020
aargtngayy tncayccnca rytntgytty aargtncarc cntggttyws nttyggnaay 1080
wsnwsncayg tngartgycc ncaycaracn ggnwsnytna cnwsntggaa ygtnwsnatg 1140
gayacncarg cncarcaryt nathytncay ttywsnwsnm gnatgcaygc nacnttywsn 1200
gcngcntggw snytnccngg nytnggncar gayacnytng tnccnccngt ntayacngtn 1260
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wsntaymgnc ayytnggnyt nytnathytn gcnytnytng cnytnytnac nytnytnggn 1380
gtngtnytng cnytnacntg ymgnmgnccn carwsnggnc cnggnccngc nmgnccngtn 1440
ytnytnytnc aygcngcnga ywsngargcn carmgnmgny tngtnggngc nytngcngar 1500
ytnytnmgng cngcnytngg nggnggnmgn gaygtnathg tngayytntg ggarggnmgn 1560
caygtngcnm gngtnggncc nytnccntgg ytntgggcng cnmgnacnmg ngtngcnmgn 1620
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gayccnmgng cngcnccnyt nytngcnytn ytncaygcng cnccnmgncc nytnytnytn 1740
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ccnmgntaym gnytnytnmg ngayytnccn mgnytnytnm gngcnytnga ygcnmgnccn 1860
ttygcngarg cnacnwsntg gggnmgnytn ggngcnmgnc armgnmgnca rwsnmgnytn 1920
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<220>
<223> zc39334

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<210> 18
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<220>
<223> zc39333

<400> 18
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<210> 19
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<220>
<223> zc40458

<400> 19
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